JOURNAL OF AGRICULTURAL RESEARCH

VOL. XX WASHINGTON, D. C., OCTOBER 15, 1920 NO. 2

INVESTIGATIONS OF THE GERMICIDAL VALUE OF SOME OF THE CHLORIN DISINFECTANTS

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SCOPE OF THE INVESTIGATION

During the great World War, which from the surgical standpoint was distinguished by the frequency and intensity of wound infections, no class of antiseptics was more extensively employed than the so-called "chlorin antiseptics." When properly used they proved to be of very great value, as may be seen by a perusal of the various publications of Carrel and his colleagues and especially the book by Carrel and Dehelly (*2*).[1]

In view of the great amount of work already done on the value of these antiseptics in surgery no attempt has been made by the writer to cover that field of work. The experiments herein described were intended to furnish information regarding the value of the chlorin antiseptics for general disinfection. The members of this group actually tested were: (1) chloramin T, (2) Dakin's solution (NaOCl), (3) eusol (HOCl), and (4) chlorin.

"Chloramin T" is the abbreviated name given by Dakin to sodium-toluene-sulphon-chloramid (*4*). It is described as a "white crystalline solid with a faint chlorous odor" containing 12.6 per cent of chlorin and readily soluble in water. The material used in the present work was obtained under the trade name "Chlorazene." Its appearance corresponds to the foregoing description, and titration of an aqueous solution with potassium iodid and sodium thiosulphate showed it to contain 25 per cent of "available chlorin," which corresponds to 12.5 per cent of actual chlorin, since according to Dakin and Dunham (*5*) one molecule of chloramin T liberates two atoms of iodin. The explanation they give is that each atom of chlorin in chloramin T is equivalent to a molecule of hypochlorous acid, which liberates two atoms of iodin from an acidified iodid solution.

[1] Reference is made by number (italic) to "Literature cited," p. 110.

The term "Dakin's solution" as used in this paper signifies a neutral solution of sodium hypochlorite. The methods of preparation were essentially those given by Dakin and Dunham (5).

The details of the method with sodium carbonate are, according to Dakin and Dunham, as follows: One hundred and forty gm. of dry sodium carbonate (Na_2CO_3), or 400 gm. of the crystallized salt, are dissolved in 10 liters of water, and 200 gm. of bleaching powder containing 24 to 28 per cent of "available chlorin" are added. The mixture is very thoroughly shaken, and after it has stood half an hour the supernatant fluid is siphoned off from the precipitate and filtered through a cotton plug or through paper. Forty gm. of boric acid are added to the filtrate, and it is then ready for use.

The details of the method with sodium carbonate and sodium bicarbonate are, according to Dakin and Dunham, as follows: Two hundred gm. of bleaching powder (containing 24 to 28 per cent of "available chlorin") are shaken well with 5 liters of water and allowed to stand for an hour or two. In a separate vessel 94 gm. of sodium carbonate and 86 gm. of sodium bicarbonate ($NaHCO_3$) are mixed with 5 liters of water, and this solution is added to the bleaching powder suspension. The mixture is well shaken and allowed to stand until the precipitate settles. The clear supernatant fluid is then siphoned off and filtered.

In actual practice the writer made the following modifications. The amount of Dakin's solution made up at any one time was always smaller than the amount indicated above, but the relative proportions of ingredients were the same. The bleaching powder was rubbed up in a mortar with a little water until it was of a creamy consistency. It was then transferred to a graduated flask or cylinder and made up to volume with more water. Dry sodium carbonate, or the solution of sodium carbonate and sodium bicarbonate, was added in accordance with the directions of Dakin and Dunham, and their further directions were followed except that instead of the clear supernatant fluid being drawn off the entire mixture was shaken up and filtered through paper. The bleaching powder used contained approximately 28 per cent of "available chlorin."

In certain experiments Dakin's solution was also prepared by the direct action of chlorin upon a solution of sodium carbonate, with the use of the apparatus devised for the purpose by the Wallace & Tiernan Co., of New York City.

The term "eusol," as employed in this paper, signifies a solution prepared from bleaching powder in aqueous solution by the addition of an equal amount of boric acid. The originators of this solution (12) describe it as a solution of hypochlorous acid, but according to Dakin and Dunham (5) the solution is alkaline to litmus and contains a balanced mixture of calcium hypochlorite and calcium borate with an undetermined amount of free hypochlorous acid.

The method of preparation as given by Dakin and Dunham is as follows: To 1 liter of water add 12.5 gm. of bleaching powder and shake vigorously. Add 12.5 gm. of powdered boric acid and shake again. Allow the mixture to stand for some hours, preferably overnight, and then filter. In actual practice the writer made the following modifications: The bleaching powder was rubbed up in a mortar with a little water until the mixture had a creamy consistency. It was then transferred to a graduated flask or cylinder, the boric acid was added, and then the amount of water necessary to make up the volume. The mixture was shaken and then usually allowed to stand about two hours before it was filtered through paper.

Chlorin was used in these experiments in the form of an aqueous solution, standardized by titration with potassium iodid and sodium thiosulphate.

Dilutions of these various disinfectants were made up for test as follows: Chloramin T dilutions were made by weighing the solid and dissolving it in the required amount of water. In certain experiments a stock solution was made and titrated with potassium iodid and sodium thiosulphate, and dilutions of the stock solution were then made so as to contain specified amounts of "available chlorin."[1] But for the most part dilutions were made up to contain a given weight of the solid chloramin T.

Dakin's solution and eusol were prepared according to the directions previously given and were then titrated with potassium iodid and sodium thiosulphate. Dilutions were then made from these original solutions so as to contain a given amount of "sodium hypochlorite" or "hypochlorous acid" for Dakin's solution and eusol, respectively. In certain experiments the dilutions were made in both instances so as to contain given amounts of "available chlorin."

It has already been noted that according to Dakin and Dunham (5) eusol contains calcium hypochlorite with an indefinite amount of free hypochlorous acid. In a similar way Dakin's solution may contain not only sodium hypochlorite but also more or less hypochlorous acid, as stated by Cullen and Austin (3). As regards "available chlorin" Rosenau (11) states that this really represents available oxygen rather than available chlorin. All three terms, however, are convenient as conventional symbols and will be so used in this paper.

It should be stated further that for the purposes of certain experiments it was necessary to modify the methods of preparing Dakin's solution and eusol materially so as to secure more concentrated solutions. In all such instances the changes made are indicated in connection with the experiments.

[1] The quotation marks used in this and the following paragraph are intended to indicate that the terms are used in a conventional way for purposes of comparison, and not in their literal sense.

EXPERIMENTS WITH STAPHYLOCOCCUS AUREUS, BACILLUS PYOCYANEUS, AND B. TYPHOSUS AS TEST ORGANISMS

The cultures used in this series of experiments were stock cultures which had been carried along in this laboratory for some time. They had been previously examined in connection with other work and had been found true to type. The cultures of *Staphylococcus aureus* and *Bacillus pyocyaneus* were originally isolated from wounds and when received in this laboratory were virulent for guinea pigs and rabbits.

The first three experiments of this series were made with chloramin T only. The results are shown in Tables I and II.

EXPERIMENT 1.—This was a preliminary experiment to test the value of chloramin T against *Staphylococcus aureus* and *Bacillus typhosus*. The technic was as follows: One-tenth cc. of 24-hour bouillon culture was mixed with 25 cc. of blood serum.[1] Then 2.5 cc. of this mixture were mixed with 2.5 cc. of a dilution of chloramin T. After exposures of one hour and two hours, respectively, subcultures were made with a 3-mm. platinum loop into tubes of standard bouillon containing enough sodium thiosulphate to neutralize the disinfectant carried over.

The number of organisms present in the test mixtures were calculated to be 1,340,000 per centimeter for *Staphylococcus aureus* and 800,000 per centimeter for *Bacillus typhosus*. Dilutions given in the table are, of course, final dilutions, and the dilutions actually made up to begin with were naturally just twice as concentrated. The results are given in Table I.

TABLE I.—*Germicidal efficiency of chloramin T against Staphylococcus aureus and Bacillus typhosus, mixed with blood serum, using equal amounts of disinfectant and of serum plus culture* [a]

EXPERIMENT 1

Concentration of chloramin T.	*Staph. aureus*		*B. typhosus.*	
	Exposed 1 hour.	Exposed 2 hours.	Exposed 1 hour.	Exposed 2 hours.
1 to 200	−	−	−	−
1 to 400	−	−	+	−
1 to 600	+	+	+	+
1 to 800	+	+	+	+

[a] + Signifies growth; −, no growth.

EXPERIMENTS 2 AND 3.—The technic used in these experiments was as follows: Each disinfectant dilution was mixed with an equal quantity of blood serum (2.5 cc. each of disinfectant dilution and serum), and to this mixture 2 drops of a 24-hour bouillon culture were added. The mixture was then vigorously shaken, and at intervals of 15 minutes, 1 hour, and 2 hours subcultures were made with a 3-mm. platinum loop

[1] Horse-blood serum was used in all experiments where blood serum is mentioned.

into tubes of standard broth. The mixtures were thoroughly shaken just before subcultures were made.

In these experiments and in all others described in this paper, the test mixtures were used at ordinary room temperatures.

The results of experiments 2 and 3 are given in Table II.

TABLE II.—*Germicidal efficiency of chloramin T against Staphylococcus aureus, Bacillus typhosus, and B. pyocyaneus when mixed with an equal quantity of blood serum before culture is added* [a]

EXPERIMENT 2

Concentration of chloramin T.	*Staph. aureus.*			*B. typhosus.*		
	Exposed 15 minutes.	Exposed 1 hour.	Exposed 2 hours.	Exposed 15 minutes.	Exposed 1 hour.	Exposed 2 hours.
1 to 200	−	−	−	−	−	−
1 to 400	+	−	−	−	−	−
1 to 600	+	+	−	+	+	+
1 to 800	+	+	+	+	+	+

EXPERIMENT 3

Concentration of chloramin T.	*B. pyocyaneus.*			*B. typhosus.*		
	Exposed 15 minutes.	Exposed 1 hour	Exposed 2 hours.	Exposed 15 minutes.	Exposed 1 hour.	Exposed 2 hours.
1 to 200	+	−	−	−	−	−
1 to 400	+	+	+	−	−	−
1 to 600	+	+	+	+	+	+
1 to 800	+	+	+	+	+	+

[a] + Signifies growth; −, no growth.

EXPERIMENTS 4 AND 5.—These were preliminary experiments with Dakin's solution, which was made up by the use of sodium carbonate alone, as described in the first of the methods of preparation previously mentioned. In order to determine what influence the boric acid exerts, two portions were tested, one with and the other without the addition of boric acid. The technic was the same as that described for experiments 2 and 3. Dilutions given are based on the amount of sodium hypochlorite. The results of these experiments are given in Table III.

The results given in Table III indicate that the boric acid adds somewhat to the germicidal power of Dakin's solution. This is probably due to the small amount of hypochlorous acid set free by the boric acid.

EXPERIMENTS 6, 7, AND 8.—These experiments were made in order to compare the germicidal powers of chloramin T and Dakin's solution against *Staphylococcus aureus*, *Bacillus pyocyaneus*, and *B. typhosus*. The technic was the same as that described for experiments 2 and 3, except for the omission of the 15-minute exposures. The results are given in Table IV.

TABLE III.—*Germicidal efficiency of Dakin's solution against Staphylococcus aureus, with and without boric acid*[a]

Concentration of NaOCl.	Without boric acid.			With boric acid.		
	Exposed 15 minutes.	Exposed 1 hour.	Exposed 2 hours.	Exposed 15 minutes.	Exposed 1 hour.	Exposed 2 hours.
1 to 100	+	−	−	−	−	−
1 to 200	+	+	−	+	−	−
1 to 400	+	+	−	+	+	−
1 to 800	+	+	+	+	+	+
EXPERIMENT 5						
1 to 100	+	−	−	−	−	−
1 to 200	+	+	−	+	−	−
1 to 400	+	+	+	+	−	−
1 to 600	+	+	+	+	+	+
1 to 800	+	+	+	+	+	+

[a] + signifies growth; −, no growth.

TABLE IV.—*Comparative germicidal efficiency of chloramin T and Dakin's solution against Staphylococcus aureus, Bacillus pyocyaneus, and B. typhosus, in the presence of 50 per cent blood serum*[a]

EXPERIMENT 6

Disinfectant and dilution.	*Staph. aureus.*		*B. pyocyaneus.*		*B. typhosus.*	
	Exposed 1 hour.	Exposed 2 hours.	Exposed 1 hour.	Exposed 2 hours.	Exposed 1 hour.	Exposed 2 hours.
Chloramin T:						
1 to 200	−	−	−	−	−	−
1 to 300	−	−	+	−	−	−
1 to 400	−	−	+	+	−	−
1 to 500	−	−	+	+	+	−
1 to 600	−	−	+	+	+	+
1 to 800	+	−	+	+	+	+
Dakin's solution:						
NaOCl 1 to 200	−	−	−	−	−	−
NaOCl 1 to 300	+	−	−	−	−	−
NaOCl 1 to 400	+	−	+	−	+	−
NaOCl 1 to 500	+	+	+	−	+	−
NaOCl 1 to 600	+	+	+	−	+	−
NaOCl 1 to 800	+	+	+	+	+	+
EXPERIMENT 7						
Chloramin T:						
1 to 200	No test.	No test.	−	−	−	−
1 to 300	...do....	...do....	−	−	−	−
1 to 400	...do....	...do....	+	−	−	−
1 to 500	+	−	+	+	−	−
1 to 600	+	−	+	+	+	+
1 to 800	+	+	No test.	No test.	+	+
1 to 1,000	+	+	...do....	...do....	No test.	No test.

[a] + signifies growth; −, no growth.

TABLE IV.—*Comparative germicidal efficiency of chloramin T and Dakin's solution against Staphylococcus aureus, Bacillus pyocyaneus, and B. typhosus, in the presence of 50 per cent blood serum*—Continued

EXPERIMENT 7—continued

Disinfectant and dilution.	*Staph. aureus.*		*B. pyocyaneus.*		*B. typhosus.*	
	Exposed 1 hour.	Exposed 2 hours.	Exposed 1 hour.	Exposed 2 hours.	Exposed 1 hour.	Exposed 2 hours.
Dakin's solution:						
NaOCl 1 to 200	+	−	No test.	No test.	No test.	No test.
NaOCl 1 to 300	+	−	−	−	−	−
NaOCl 1 to 400	+	+	−	−	+	−
NaOCl 1 to 500	+	+	+	−	+	−
NaOCl 1 to 600	+	+	+	−	+	−
NaOCl 1 to 800	No test.	No test.	+	+	+	+
NaOCl 1 to 1,000	...do....	...do....	+	+	+	+

EXPERIMENT 8

Chloramin T:						
1 to 200	No test.	No test.	−	−	−	−
1 to 300	...do....	...do....	+	−	−	−
1 to 400	−	−	+	+	+	+
1 to 500	−	−	+	+	+	+
1 to 600	+	−	+	+	+	+
1 to 800	+	+	No test.	No test.	No test.	No test.
1 to 1,000	+	+	...do....	...do....	...do....	Do.
Dakin's solution:						
NaOCl 1 to 200	+	−	...do....	...do....	−	−
NaOCl 1 to 300	+	−	−	−	No test.	No test.
NaOCl 1 to 400	+	−	+	−	+	−
NaOCl 1 to 500	+	−	+	−	No test.	No test.
NaOCl 1 to 600	+	+	+	−	+	−
NaOCl 1 to 800	+	+	+	+	+	+
NaOCl 1 to 1,000	No test.	No test	+	+	+	+

EXPERIMENTS 9, 10, AND 11.—These experiments were undertaken to determine the efficiency of chloramin T and Dakin's solution against *Staphylococcus aureus*, *Bacillus pyocyaneus*, and *B. typhosus* without the addition of blood serum. They are in contrast with the three preceding experiments, in which 50 per cent of blood serum was used.

The technic was as follows: Two drops of a 24-hour bouillon culture were added to 5 cc. of disinfectant, and the mixture was well shaken. After intervals of 1 hour and 2 hours, respectively, the mixtures were again shaken and subcultures were made with a 3-mm. platinum loop into tubes of standard bouillon containing enough sodium thiosulphate to neutralize the disinfectant carried over. The results are given in Table V.

In the results shown in Tables IV and V there is seen evidence of what may be called "selective" action on the part of the two disinfectants tested. For instance, the amount of chloramin T required to kill *Staphylococcus aureus* is very much less than that required to kill *Bacillus pyocyaneus*. In like manner in the presence of blood serum it requires more

Dakin's solution to kill *Staph. aureus* than to kill *B. pyocyaneus* or *B. typhosus* under like conditions. A comparison of the two disinfectants shows that Dakin's solution is more effective than chloramin T against *B. pyocyaneus*, while chloramin T is more effective than sodium hypochlorite against *Staph. aureus*.

TABLE V.—*Comparative germicidal efficiency of chloramin T and Dakin's solution against Staphylococcus aureus, Bacillus pyocyaneus, and B. typhosus, without addition of blood serum 1*[a]

EXPERIMENT 9

Disinfectant and dilution.	*Staph. aureus.*		*B. pyocyaneus.*		*B. typhosus.*	
	Exposed 1 hour.	Exposed 2 hours.	Exposed 1 hour.	Exposed 2 hours.	Exposed 1 hour.	Exposed 2 hours.
Chloramin T:						
1 to 1,000	−	−	−	−	−	−
1 to 10,000	−	−	+	+	+	−
1 to 25,000	−	−	+	+	+	−
1 to 50,000	+	+	+	+	+	+
1 to 100,000	+	+	+	+	+	+
Dakin's solution:						
NaOCl 1 to 1,000	−	−	−	−	−	−
NaOCl 1 to 10,000	−	−	−	−	−	−
NaOCl 1 to 25,000	−	−	−	−	−	−
NaOCl 1 to 50,000	−	−	−	−	+	+
NaOCl 1 to 100,000	+	+	+	+	+	+

EXPERIMENT 10

Chloramin T:						
1 to 1,000	−	−	−	−	−	−
1 to 10,000	−	−	+	+	+	−
1 to 30,000	−	−	+	+	+	+
1 to 50,000	+	+	+	+	+	+
1 to 100,000	+	+	+	+	+	+
Dakin's solution:						
NaOCl 1 to 1,000	−	−	−	−	−	−
NaOCl 1 to 10,000	−	−	−	−	−	−
NaOCl 1 to 30,000	−	−	+	+	−	−
NaOCl 1 to 50,000	+	−	+	+	+	+
NaOCl 1 to 100,000	+	−	+	+	+	+

EXPERIMENT 11

Chloramin T:						
1 to 1,000	−	−	+	−	−	−
1 to 10,000	−	−	+	+	−	−
1 to 30,000	−	−	+	+	+	−
1 to 50,000	−	−	+	+	+	−
1 to 100,000	+	+	+	+	+	+
Dakin's solution:						
NaOCl 1 to 1,000	−	−	−	−	−	−
NaOCl 1 to 10,000	−	−	−	−	−	−
NaOCl 1 to 30,000	−	−	+	+	−	−
NaOCl 1 to 50,000	−	−	+	+	−	−
NaOCl 1 to 100,000	+	+	+	+	+	+

[a] + signifies growth; −, no growth.

EXPERIMENTS 12 AND 13.—These experiments were made for the purpose of comparing the germicidal activity of chloramin T, Dakin's solution, and eusol against *Staphylococcus aureus*, *Bacillus pyocyaneus*, and *B. typhosus*.

The technic was as follows: Each dilution of the disinfectant was mixed with an equal amount of a 24-hour culture of the test organism, and the mixture was thoroughly shaken. After intervals of 10 minutes and 30 minutes, respectively, the mixtures were again shaken, and subcultures were made with a 3-mm. platinum loop into tubes of standard bouillon containing enough sodium thiosulphate to neutralize the disinfectant carried over. The amounts of culture and disinfectant used were 2.5 cc. of each. For purposes of comparison, tests were made with mercuric chlorid, and in these tests sodium sulphid was used to neutralize the disinfectant carried over. The results are given in Table VI.

TABLE VI.—*Comparative germicidal efficiency of chloramin T, Dakin's solution, eusol, and mercuric chlorid against Staphylococcus aureus, Bacillus pyocyaneus, and B. typhosus* [a]

EXPERIMENT 12

Disinfectant and dilution.	*Staph. aureus.*		*B. pyocyaneus.*		*B. typhosus.*	
	Exposed 10 minutes.	Exposed 30 minutes.	Exposed 10 minutes.	Exposed 30 minutes.	Exposed 10 minutes.	Exposed 30 minutes.
Chloramin T:						
1 to 1,000.	−	−	+	−	−	−
1 to 2,000.	−	−	+	+	−	−
Dakin's solution:						
NaOCl 1 to 2,000.	−	−	−	−	−	−
Eusol:						
HOCl 1 to 2,000.	−	−	−	−	−	−
Mercuric chlorid:						
1 to 2,000.	−	−	−	−	−	−

EXPERIMENT 13

Chloramin T:						
1 to 1,000.	−	−	+	−	−	−
1 to 2,000.	−	−	+	+	−	−
Dakin's solution:						
NaOCl 1 to 2,000.	−	−	−	−	−	−
NaOCl 1 to 4,000.	−	−	−	−	−	−
Eusol:						
HOCl 1 to 2,000.	−	−	−	−	−	−
HOCl 1 to 4,000.	−	−	−	−	−	−
Mercuric chlorid:						
1 to 2,000.	+	+	−	−	+	−
1 to 4,000.	+	+	−	−	+	−

[a] + signifies growth; −, no growth.

EXPERIMENTS 14 AND 15.—In these experiments the same disinfectants were compared as in experiments 12 and 13, but with the addition of blood serum. The technic was the same also, except that a mixture of equal parts of blood serum and culture was used instead of culture alone. The results are given in Table VII.

TABLE VII.—*Comparative germicidal efficiency of chloramin T, Dakin's solution, eusol, and mercuric chlorid against Staphylococcus aureus, Bacillus pyocyaneus, and B. typhosus in the presence of 25 per cent blood serum*[a]

EXPERIMENT 14

Disinfectant and dilution.	*Staph. aureus.*		*B. pyocyaneus.*		*B. typhosus.*	
	Exposed 10 minutes	Exposed 30 minutes	Exposed 10 minutes.	Exposed 30 minutes.	Exposed 10 minutes.	Exposed 30 minutes.
Chloramin T:						
1 to 500	−	−	+	−	−	−
1 to 1,000	−	−	+	+	+	+
Dakin's solution:						
NaOCl 1 to 2,000	−	−	−	−	−	−
Eusol:						
HOCl 1 to 2,000	−	−	−	−	−	−
Mercuric chlorid:						
1 to 2,000	+	−	+	−	+	−

EXPERIMENT 15

Chloramin T:						
1 to 1,000	−	−	+	+	+	+
1 to 2,000	+	−	+	+	+	+
Dakin's solution:						
NaOCl 1 to 2,000	+	−	−	−	+	−
NaOCl 1 to 4,000	+	−	+	+	+	−
Eusol:						
HOCl 1 to 2,000	−	−	−	−	−	−
HOCl 1 to 4,000	−	−	−	−	−	−
Mercuric chlorid:						
1 to 2,000	+	+	−	−	+	−
1 to 4,000	+	+	−	−	+	−

[a] + signifies growth; − no growth.

The four experiments shown in Tables VI and VII show that eusol is decidedly superior to chloramin T, Dakin's solution, and mercuric chlorid, especially in the presence of blood serum. Unfortunately, however, eusol is very unstable and for that reason is not reliable, since it is impossible in practice to count on its containing any uniform amount of active material. In the tests reported here the eusol was made up just before the test and was used as soon as possible, but observations which were made in connection with chemical work upon these various disinfectants would tend to show that there was probably a perceptible loss of strength even in the time required for a test.

EXPERIMENTS WITH ANTHRAX SPORES

The experiments upon anthrax spores were performed by the Hill (6) "rod" method, with some modifications. The method as modified is as follows: Glass rods $^3/_{19}$-inch in diameter and 8 inches long are etched at one end, the etched portion being about 1 inch long. Cotton is wrapped

about the rods near the end not etched, and the rods are thrust into test tubes so as to engage the cotton in the mouth of the tube. The tubes containing the rods are sterilized by dry heat (150° C.) for 1 hour or more. In making tests the rods are removed from the tubes and the etched portions are dipped into a suspension made from a culture of the organism to be tested. They are then replaced in the tubes and dried in the incubator for one hour.

Rods so infected are transferred to test tubes containing the disinfectant to be tested, the amount of disinfectant being sufficient to cover all the infected portion of the rod. They are exposed to the action of the disinfectant for varying lengths of time. After exposure the rods are washed with sterile water in order to remove traces of the disinfectant and are then transferred to tubes containing bouillon or agar, which are incubated for at least 48 hours at 37.5° C. The suspension used in infecting the rods is made from the surface growth on an agar tube by rubbing up in several cubic centimeters of sterile water enough of the growth to give a suspension of approximately the same density as a 24-hour bouillon culture of *Bacillus typhosus*. For an organism that does not bear spores the culture should be 24 hours old, while for spore-bearing organisms cultures 1 to 2 weeks old are usually the most suitable.

In making tests with a disinfectant containing mercury it is advisable to dip the rods into a saturated solution of hydrogen sulphid or an aqueous solution of some sulphid before placing them in subculture tubes. In this connection it should be mentioned that media of acid reaction have been found to exert an inhibitory action upon the growth of *Bacillus anthracis* after exposure to disinfectants. For that reason the media used in these experiments have been neutral or slightly alkaline.

EXPERIMENTS 16 AND 17.—In these experiments chloramin T was tested in varying concentrations, both in water and in 50 per cent blood serum. A sterile 10 per cent solution of sodium thiosulphate was used for washing the rods before placing them in subculture tubes of exactly neutral broth. The results are given in Table VIII.

TABLE VIII.—Germicidal efficiency of chloramin T against anthrax spores, with and without the addition of blood serum [a]

EXPERIMENT 16

Concentration of chloramin T.	Amount of serum.	Exposed 2 hours.	Exposed 4 hours.	Exposed 24 hours.
Per cent.				
10	None	+	+	−
10	50 per cent	+	−	−
5	None	+	+	−
5	50 per cent	−	−	−
1	None	+	+	−
1	50 per cent	+	+	−
Control rod			+	+

[a] + signifies growth; −, no growth.

TABLE VIII.—*Germicidal efficiency of chloramin T against anthrax spores, with and without the addition of blood serum*—Continued.

EXPERIMENT 17

Concentration of chloramin T.	Amount of serum.	Exposed 2 hours.	Exposed 4 hours.	Exposed 24 hours.
Per cent.				
5	None	+	+	−
5	50 per cent	+	−	−
1	None	+	+	−
1	50 per cent	+	+	−
0.5	None	+	+	+
0.5	50 per cent	+	+	+
Control rod			+	+

EXPERIMENT 18.—This experiment was undertaken in order to compare chloramin T and Dakin's solution. The results are given in Table IX.

TABLE IX.—*Comparative germicidal efficiency of chloramin T and Dakin's solution against anthrax spores, with and without the addition of blood serum* [a]

EXPERIMENT 18

Disinfectant and dilution.	Amount of serum.	Exposed 2 hours.	Exposed 4 hours.	Exposed 24 hours
Chloramin T:				
2 per cent	None	+	+	+
Do	50 per cent	+	+	−
1 per cent	None	+	+	+
Do	50 per cent	+	+	−
0.5 per cent	None	+	+	+
Do	50 per cent	+	+	+
Dakin's solution:				
NaOCl 2 per cent	None	−	−	−
Do	50 per cent	+	+	−
NaOCl 1 per cent	None	−	−	−
Do	50 per cent	+	+	−
NaOCl 0.5 per cent	None	−	−	−
Do	50 per cent	+	+	+
Control rod		+	+	+

[a] + signifies growth; −, no growth

EXPERIMENT 19.—In this experiment eusol was used, the chlorin being estimated as HOCl. In the dilutions there was approximately 0.128 gm. HOCl per 100 cc. The results are given in Table X.

TABLE X.—*Germicidal efficiency of eusol against anthrax spores* [a]

EXPERIMENT 19

Time of exposure.	No serum.	50 per cent serum.
30 minutes	+	+
1 hour	+	+
2 hours	+	+
3 hours	+	+
4 hours	−	+
5 hours	−	+
24 hours	−	+

[a] + signifies growth; −, no growth.

EXPERIMENTS 20 AND 21.—In these experiments comparison was made between chloramin T, Dakin's solution, eusol, and mercuric chlorid. The results are given below in Table XI. The results with mercuric chlorid are included for comparison.

TABLE XI.—*Comparative germicidal efficiency of chloramin T, Dakin's solution, eusol, and mercuric chlorid against anthrax spores, with and without blood serum* [a]

EXPERIMENT 20

Disinfectant and dilution.	Amount of serum.	Exposed 2 hours.	Exposed 4 hours.	Exposed 1 day.	Exposed 2 days.	Exposed 4 days.
Chloramin T:						
1 to 100	None	+	+	+	−	−
1 to 100	50 per cent	+	+	+	+	+
1 to 200	None	+	+	+	−	−
1 to 200	50 per cent	+	+	+	+	+
Dakin's solution:						
NaOCl 1 to 100	None	−	−	−	−	−
NaOCl 1 to 100	50 per cent	+	+	+	+	+
NaOCl 1 to 200	None	−	−	−	−	−
NaOCl 1 to 200	50 per cent	+	+	+	+	+
Eusol:						
HOCl 1 to 200	None	−	−	−	−	−
HOCl 1 to 200	50 per cent	+	+	+	−	−
HOCl 1 to 400	None	−	−	−	−	−
HOCl 1 to 400	50 per cent	+	+	+	+	+
Mercuric chlorid:						
1 to 2,000	None	+	−	−	−	−
1 to 2,000	50 per cent	+	+	+	+	+

EXPERIMENT 21

Chloramin T:						
5 per cent	25 per cent		+	−	−	−
5 per cent	50 per cent		+	−	−	−
5 per cent	None	+	+	+	−	−
Dakin's solution:						
NaOCl 1 per cent	25 per cent		+	+	+	.....
NaOCl 1 per cent	50 per cent		.....	+	+	+
NaOCl 1 per cent	None	−	−	−	−	−
Eusol:						
HOCl 0.75 per cent	25 per cent		−	−	−	.....
HOCl 0.75 per cent	50 per cent		.....	+	−	−
HOCl 0.75 per cent	None	−	−	−	.....	.....

[a] + signifies growth; − no growth.

It should be noted here that in the experiments upon anthrax spores the strength of NaOCl and HOCl required was in most instances greater than that obtained by preparing Dakin's solution and eusol by the methods described at the beginning of this paper. So in these instances the solutions were made with less water in proportion to the other ingredients. Aside from this change the methods of preparation were the same.

The experiments upon anthrax spores indicate that if comparison is made on the basis of weight of chloramin T against weight of chlorin

as NaOCl or HOCl, in Dakin's solution and eusol, respectively, chloramin T must be regarded as less efficient than Dakin's solution or eusol against naked spores. In the presence of blood serum it is more or less equal to Dakin's solution, while eusol seems to be superior to both chloramin T and Dakin's solution. Comparison on the basis of "available" chlorin would, of course, be much more favorable to chloramin, since it contains only 25 per cent available chlorin, or 12½ per cent actual chlorin.

It is interesting to note that in experiments 16, 17, 18, and 21 chloramin T was more efficient against anthrax spores in the presence of blood serum than in the absence of serum. In experiments 16 and 17 this is true only for the stronger dilutions (10 per cent and 5 per cent) and is not true for the lowest dilution (1 per cent). In experiment 18 it is true for 2 per cent and 1 per cent dilutions after 24 hours, but in experiment 20 with dilutions of 1 to 100 and 1 to 200 and exposures of 2 days there is greater efficiency without serum than with it. Experiment 21 confirms the results obtained in experiments 16 and 17 with a 5 per cent dilution.

These experiments also seem more or less at variance with the widely expressed opinion that chlorin compounds rapidly lose their activity and soon become inert, especially in the presence of organic matter. For example, in experiment 20, HOCl 1 to 200 did not destroy anthrax spores until after an exposure of 2 days, the 4-day result serving as a control to show the correctness of the result.

This usually accepted opinion is controverted by Rideal (9), who as the result of his own experiments concludes that—

> chlorin has a disinfectant value out of all proportion to that which would be expected from the hitherto accepted theories, even in the presence of a chemical excess of organic matter in certain forms.

The explanation which he gives is that the disinfecting action of chlorin is not due merely to oxidation but also to the action of products formed by its substitution for hydrogen in ammonia and organic compounds.

EXPERIMENTS WITH BACILLUS TUBERCULOSIS

In experiments upon the tubercle bacillus the method was as follows Two and one-half cc. of disinfectant dilution were added to 2½ cc. of a suspension of culture (or a mixture of such suspension with an equal quantity of horse-blood serum), and they were mixed thoroughly by vigorous shaking. The suspension was made by rubbing up in sterile distilled water enough of the surface growth from a bouillon culture to give a suspension whose density was approximately equal to that of a 24-hour culture of *Bacillus typhosus*. After an exposure of 10 minutes enough sterile sodium thiosulphate solution (or sodium sulphid where mercuric chlorid was used) was added to insure complete neutralization, and finally 1 cc. of each neutralized test mixture was injected subcutaneously into a guinea pig.

This technic was used in making a number of comparative experiments with chloramin T, Dakin's solution, eusol, and mercuric chlorid. The results are given in Tables XII and XIII.

TABLE XII.—*Comparative germicidal efficiency of chloramin T, Dakin's solution, eusol, and mercuric chlorid against Bacillus tuberculosis, with and without the addition of 25 per cent blood serum*

EXPERIMENT 22

Disinfectant and dilution.	Amount of serum.	Guinea pig No.	Result.	Autopsy.
Chloramin T:				
1 to 1,000	None	53414	Died	Typical lesions.
Do	25 per cent	53415	do	Do.
Dakin's solution:				
NaOCl 1 to 1,000	None	53416	do	Do.
Do	25 per cent	53417	do	Do.
Eusol:				
HOCl 1 to 1,000	None	53418	Killed[a]	
Do	25 per cent	53419	Died	Do.
Mercuric chlorid:				
1 to 1,000	None	53420	do	Do.
Do	25 per cent	53421	do	Do.
Tubercle bacillus suspension.		53422	do	Do.
Do	+ serum	53423	do	Do.

EXPERIMENT 23

Chloramin T:				
1 to 100	None	54023	Died	Typical lesions.
Do	25 per cent	54024	do	Do.
Dakin's solution:				
NaOCl 1 to 500	None	54025	do	Do.
Do	25 per cent	54026	do	Do.
Eusol:				
HOCl 1 to 250	do	54027	do	Do.
HOCl 1 to 500	do	54028	do	Do.
Mercuric chlorid:				
1 to 500	None	54031	Killed[b]	
Do	25 per cent	54032	Died	Do.
Tubercle bacillus suspension.		54033	do	Do.
Do	+ serum	54034	do	Do.

[a] Killed after 33 days; perfectly normal.

[b] Killed after 2 months; perfectly normal.

EXPERIMENTS 24 AND 25.—In these experiments the disinfectants were compared on the basis of the available chlorin contained, so these experiments are grouped by themselves in Table XIII.

In the experiments upon *Bacillus tuberculosis*, as in experiments upon anthrax spores, it was necessary to use Dakin's solution and eusol of greater strength, and, as before, this result was obtained by lessening the amount of water while the other ingredients and the method of manufacture remained unchanged.

TABLE XIII.—*Comparative germicidal efficiency of chloramin T, Dakin's solution, eusol, and mercuric chlorid against Bacillus tuberculosis, with dilutions based on available chlorin*

EXPERIMENT 24

Disinfectant and dilution.	Available chlorin.	Amount of serum.	Guinea pig No.	Result.	Autopsy.
Chloramin T	1 to 200	None.	54549	Died	Typical lesions.
Do	1 to 200	25 per cent.	54550	do	Do.
Eusol	1 to 200	None.	54551	Killed[a]	Do.
Do	1 to 200	25 per cent.	54552	Died	Do.
Dakin's solution	1 to 200	None.	54555	do	Not tuberculous.[b]
Do	1 to 200	25 per cent.	54556	do	Typical lesions.
Mercuric chlorid:					
1 to 500		None.	54557	Killed[a]	Normal.
Do		25 per cent.	54558	Died	Typical lesions.
Tubercle bacillus suspension.		None.	54559	do	Do.
Do		25 per cent.	54560	do	Do.

EXPERIMENT 25

Chloramin T	1 to 200	None.	55552	Died	Typical lesions.
Do	1 to 200	25 per cent.	55553	do	Do.
Eusol	1 to 300	None.	55554	do	Not tuberculous.[c]
Do	1 to 300	25 per cent.	55555	do	Typical lesions.
Dakin's solution	1 to 200	None.	55558	do	Not tuberculous.[d]
Do	1 to 200	25 per cent.	55559	do	Typical lesions.
Mercuric chlorid:					
1 to 500		None.	55560	Killed[e]	Normal.
Do		25 per cent.	55561	Died	Typical lesions.
Tubercle bacillus suspension.		None.	55562	do	Do.
Do		25 per cent.	55563	do	Do.

[a] Killed after 10 weeks.
[b] Died after 7 weeks of an intercurrent pneumonia.
[c] Died after 2 months; no lesions observed; death probably due to scurvy.
[d] Died after 1 month of an intercurrent pneumonia.
[e] Killed after 2 months; perfectly normal.

The results of the experiments upon the tubercle bacillus would seem to indicate that the chlorin compounds are entirely inefficient so far as that organism is concerned. These are the results to be expected in view of the use of antiformin for isolating tubercle bacilli.[1]

CARBOLIC-ACID COEFFICIENTS OF THE CHLORIN ANTISEPTICS

The results here given are those of a large number of tests made by the Rideal-Walker method (*10*), modified only as stated below. Aside from the use of *Staphylococcus aureus* and *Bacillus pyocyaneus* as test organisms in addition to *B. typhosus*, the only modifications were the use of bacto-peptone instead of Witte's peptone and a relaxation of the rule that coefficients are to be deduced only where there is life after 5 minutes and death after 7½ minutes.

On account of variation in the resistance of the cultures, especially *Staphylococcus aureus* and *Bacillus pyocyaneus*, it was inconvenient to

[1] Amounts actually found inefficient were as follows: Chloramin T, 1 to 50; eusol, 0.5 per cent; and Dakin's solution, 0.5 per cent.

adhere strictly to the rule; and coefficients were deduced at any time within the 15-minute period, except that no coefficient was deduced unless there was growth in the phenol subculture tubes after both 2½ and 5 minutes' exposure. This is really only a return to previous practice (7), and the results obtained are sufficiently accurate for all practical purposes.

In all these tests, dilutions were based on the amount of available chlorin; and it should, therefore, be understood that the coefficients are really, so to speak, those of available chlorin as it is present in chloramin T, eusol, Dakin's solution, and chlorin water.

It should also be noted that in order to make the original solutions more nearly equal in chlorin content the amount of bleaching powder in proportion to water was the same for eusol as for Dakin's solution. The amount used was 5 gm. to 250 cc., which follows the usual proportion for Dakin's solution but varies from the usual proportion for eusol. These original solutions were then diluted with distilled water to obtain the desired amounts of available chlorin in the various dilutions.

The results are summarized in Table XIV, the successive figures from top to bottom in each column being coefficients obtained at various times. It will be noted that they do not always agree perfectly, but they are not offered as examples of accuracy. On the contrary, they are to be considered as approximate values to be taken for what they are worth as illustrations of the general principles of selective action already shown to a greater or less degree in previous experiments.

TABLE XIV.—*Coefficients of chloramin T, Dakin's solution, eusol, and chlorin water, based on the content of available chlorin*

Chloramin T.			Dakin's solution.			Eusol.			Chlorin water.		
Staphylococcus aureus.	*Bacillus pyocyaneus.*	*Bacillus typhosus.*	*Staphylococcus aureus.*	*Bacillus pyocyaneus.*	*Bacillus typhosus.*	*Staphylococcus aureus.*	*Bacillus pyocyaneus.*	*Bacillus typhosus.*	*Staphylococcus aureus.*	*Bacillus pyocyaneus.*	*Bacillus typhosus.*
114	8.3	66.6	57	66.6	66.6	114	120	100	92.3	80	88
92.3	8.8	66.6	57	55.5	66.6	92.3	120	111	92.3	80	80
92.3	8.3	66.6	57	66.6	66.6	92.3	120	111	92.3	80	80
92.3	.....	.....	.....	66.6	.....	92.3	.....	111	.....	.....	.....
92.3	.....	.....	.....	55.5	.....	.....	.....	.....	.....	.....	.....
.....	.....	.....	.....	66.6	.....	.....	.....	.....	.....	.....	.....

In connection with preceding experiments solutions of chlorin T, Dakin's solution, and eusol were kept in a dark closet at room temperature, and titrations were made at intervals to detect any changes that might occur. It was found that Dakin's solution and solutions of chloramin T will keep for a month or more without any great loss of available chlorin; while, on the other hand, eusol deteriorates rapidly, there being a noticeable change even within 24 hours. For example, in one instance a sample of Dakin's solution showed only about 10 per cent loss after standing 6

months, while a sample of eusol lost 10 per cent of its available chlorin in 24 hours.

In view of the instability of eusol an attempt was made to secure a more stable product by reducing the amount of boric acid, and it was found that by reducing the proportions from equal parts of bleaching powder and boric acid to 10 parts of bleaching powder and 3 parts of boric acid a product was obtained which was fully as stable as Dakin's solution. This modified eusol was tested by the carbolic-acid coefficient method in comparison with the regular eusol. The results are given in Table XV. Eusol made by the original formula is designated as eusol I, while that made by the modified formula is designated as eusol II.

TABLE XV.—*Carbolic-acid coefficients of eusol I (original formula) and eusol II (modified formula), based on available chlorin*

Date.	Solution.	Coefficient with *Staphylococcus aureus.*	Coefficient with *Bacillus pyocyaneus.*	Coefficient with *Bacillus typhosus.*
Feb. 4	Eusol I	92.3	120	111
Do	Eusol II	77	80	66.6
Feb. 7	Eusol I	92.3	120	100
Do	Eusol II	77	80	66.6

According to the results of these comparative tests it would seem that eusol I is superior to eusol II in germicidal efficiency.

INFLUENCE OF AMMONIA UPON THE GERMICIDAL EFFICIENCY OF CHLORIN DISINFECTANTS

It has been shown by Race (*8*) and Rideal (*9*) that the addition of ammonia to electrolytic hypochlorite solutions greatly increases their germicidal efficiency. Their explanation of this increase is that it is due to the formation of chloramin (NH_2Cl). The experiments here discussed were intended to verify these findings by the use of methods similar to those employed in the experiments already discussed, without attempting to ascertain the cause of the increased germicidal value.

The method first used was the Rideal-Walker method (*10*), modified by the use of an unadjusted culture medium as recommended by the American Public Health Association Committee on Standard Methods of Examining Disinfectants (*11*). The method was also modified by deducing coefficients at times other than 7½ minutes, and in many instances no coefficient was obtained.

By the use of this method experiments were first made upon Dakin's solution, prepared from bleaching powder by the use of sodium carbonate and bicarbonate as previously described. Ammonia was added so as to furnish a molecular equivalent to the sodium hypochlorite of the Dakin's solution. Experiment 26 (Table XVI) shows the comparative results with no organic matter added, and experiments 27 and 28 (Table XVI) show the results with blood serum added.

TABLE XVI.—*Effect of addition of ammonia upon germicidal activity of Dakin's solution against Bacillus typhosus*[a]

EXPERIMENT 26

WITHOUT AMMONIA; NO BLOOD SERUM ADDED

Concentration of NaOCl.	Exposed 2½ minutes.	Exposed 5 minutes.	Exposed 7½ minutes.	Exposed 10 minutes.	Exposed 12½ minutes.	Exposed 15 minutes.	
1 to 2,000	−	−	−	−	−	−	
1 to 4,000	+	+	+	−	−	−	Coefficient 57, $\frac{4,000}{70} = 57$.
1 to 6,000	+	+	+	+	+	+	
1 to 8,000	+	+	+	+	+	+	
Phenol 1 to 70	+	+	+	−	−	−	

WITH AMMONIA; NO BLOOD SERUM ADDED

1 to 6,000	+	+	−	−	−	−	
1 to 8,000	+	+	+	−	−	−	Coefficient 86, $\frac{6,000}{70} = 86$.
1 to 10,000	+	+	+	−	−	−	
1 to 12,000	+	+	+	+	−	−	
Phenol 1 to 70	+	+	−	−	−	−	

EXPERIMENT 27

WITHOUT AMMONIA; 5 PER CENT BLOOD SERUM ADDED

1 to 500	+	+	+	+	+	+
1 to 1,000	+	+	+	+	+	+
1 to 2,000	+	+	+	+	+	+
1 to 4,000	+	+	+	+	+	+
Phenol 1 to 80[b]	+	+	+	+	+	−

WITH AMMONIA; 5 PER CENT BLOOD SERUM ADDED

1 to 500	−	−	−	−	−	−
1 to 1,000	−	−	−	−	−	−
1 to 2,000	−	−	−	−	−	−
1 to 4,000	+	−	−	−	−	−
Phenol 1 to 80[b]	+	+	+	+	−	−

EXPERIMENT 28

WITH AMMONIA; 10 PER CENT BLOOD SERUM ADDED

1 to 500	−	−	−	−	−	−
1 to 1,000	+	−	−	−	−	−
1 to 2,000	+	+	+	−	−	−
1 to 4,000	+	+	+	+	+	+
Phenol 1 to 70[b]	+	−	−	−	−	−

WITH AMMONIA; 50 PER CENT BLOOD SERUM ADDED

1 to 500	+	+	+	+	+	−
1 to 1,000	+	+	+	+	+	+
1 to 1,500	+	+	+	+	+	+
1 to 2,000	+	+	+	+	+	+
Phenol 1 to 70[b]	+	−	−	−	−	−

[a] + signifies growth; −, no growth. [b] No blood serum added.

The experiments shown above in Table XVI indicate that the addition of a molecular equivalent of ammonia to Dakin's solution not only greatly increases its germicidal value against "naked" bacteria, but, to a large extent, prevents depreciation of germicidal value due to the addition of blood serum.

In Table XVII there are shown the results of a number of experiments upon chlorin water, with and without a molecular equivalent of ammonia.

TABLE XVII.—*Effect of ammonia upon the germicidal activity of chlorin in aqueous solution against Bacillus typhosus* [a]

EXPERIMENT 29

WITHOUT AMMONIA

Concentration of chlorin.	Exposed 2½ minutes.	Exposed 5 minutes.	Exposed 7½ minutes.	Exposed 10 minutes.	Exposed 12½ minutes.	Exposed 15 minutes.
1 to 4,000	−	−	−	−	−	−
1 to 8,000	−	−	−	−	−	−
1 to 12,000	−	−	−	−	−	−
1 to 16,000	+	+	−	−	−	−
Phenol 1 to 80	+	+	+	−	−	−

WITH AMMONIA

1 to 4,000	−	−	−	−	−	−
1 to 8,000	+	−	−	−	−	−
1 to 12,000	+	+	+	−	−	−
1 to 16,000	+	+	+	+	+	−
Phenol 1 to 80	+	+	+	−	−	−

EXPERIMENT 30

WITHOUT AMMONIA; 10 PER CENT BLOOD SERUM ADDED

1 to 500	−	−	−	−	−	−
1 to 1,000	+	−	−	−	−	−
1 to 2,000	+	+	+	+	+	+
1 to 4,000	+	+	+	+	+	+
Phenol 1 to 80 [b]	+	+	+	+	+	−

WITH AMMONIA; 10 PER CENT BLOOD SERUM ADDED

1 to 1,000	−	−	−	−	−	−
1 to 2,000	−	−	−	−	−	−
1 to 4,000	−	−	−	−	−	−
1 to 6,000	−	−	−	−	−	−
Phenol 1 to 80 [b]	−	−	−	−	−	−

[a] + signifies growth; −, no growth.

[b] No blood serum added.

The experiments shown in Table XVII indicate that the addition of a molecular equivalent of ammonia to chlorin water decreases rather than increases the germicidal value of the chlorin in the absence of organic matter, but it does tend to prevent depreciation of germicidal activity on the addition of blood serum.

The experiments shown in Table XVIII were designed to determine the optimum amount of ammonia.

TABLE XVIII.—*Effect of varying amounts of ammonia upon the germicidal value of chlorin in aqueous solution* [a]

EXPERIMENT 31

WITH MOLECULAR EQUIVALENT OF AMMONIA

Concentration of chlorin.	Exposed 2½ minutes.	Exposed 5 minutes.	Exposed 7½ minutes.	Exposed 10 minutes.	Exposed 12½ minutes	Exposed 15 minutes.
1 to 5,000	−	−	−	−	−	−
1 to 10,000	+	+	−	−	−	−
1 to 15,000	+	+	+	+	+	+
1 to 20,000	+	+	+	+	+	+
Phenol 1 to 80	+	+	+	−	−	−

WITH ONE-HALF MOLECULAR EQUIVALENT OF AMMONIA

1 to 5,000	−	−	−	−	−	−
1 to 10,000	−	−	−	−	−	−
1 to 15,000	−	−	−	−	−	−
1 to 20,000	+	+	+	+	+	+
Phenol 1 to 80	+	+	+	+	−	−

EXPERIMENT 32

WITH MOLECULAR EQUIVALENT OF AMMONIA

1 to 5,000	−	−	−	−	−	−
1 to 10,000	+	−	−	−	−	−
1 to 15,000	+	+	+	+	−	−
1 to 20,000	+	+	+	+	+	+
Phenol 1 to 80	+	+	−	−	−	−

WITH TWO MOLECULAR EQUIVALENTS OF AMMONIA

1 to 5,000	+	+	+	+	−	−
1 to 10,000	+	+	+	+	+	+
1 to 15,000	+	+	+	+	+	+
1 to 20,000	+	+	+	+	+	+
Phenol 1 to 80	+	+	−	−	−	−

[a] + signifies growth −, no growth.

TABLE XVIII.—*Effect of varying amounts of ammonia upon the germicidal value of chlorin in aqueous solution*—Continued

EXPERIMENT 33

WITH ONE-HALF MOLECULAR EQUIVALENT OF AMMONIA

Concentration of chlorin.	Exposed 2½ minutes.	Exposed 5 minutes.	Exposed 7½ minutes.	Exposed 10 minutes.	Exposed 12½ minutes.	Exposed 15 minutes.
1 to 10,000	−	−	−	−	−	−
1 to 15,000	+	+	+	+	+	−
1 to 20,000	+	+	+	+	+	+
1 to 25,000	+	+	+	+	+	+
Phenol 1 to 80	+	+	−	−	−	−

WITH ONE-FOURTH MOLECULAR EQUIVALENT OF AMMONIA

1 to 10,000	−	−	−	−	−	−
1 to 15,000	+	+	+	+	+	−
1 to 20,000	+	+	+	+	+	+
1 to 25,000	+	+	+	+	+	+
Phenol 1 to 80	+	+	−	−	−	−

EXPERIMENT 34

WITH MOLECULAR EQUIVALENT OF AMMONIA; 10 PER CENT BLOOD SERUM ADDED

1 to 1,000	+	+	+	+	−	−
1 to 2,000	+	+	+	+	+	+
1 to 4,000	+	+	+	+	+	+
1 to 6,000	+	+	+	+	+	+
Phenol 1 to 80	+	+	−	−	−	−

WITH ONE-HALF MOLECULAR EQUIVALENT OF AMMONIA; 10 PER CENT BLOOD SERUM ADDED

1 to 1,000	+	−	−	−	−	−
1 to 2,000	+	+	+	+	+	+
1 to 4,000	+	+	+	+	+	+
1 to 6,000	+	+	+	+	+	+
Phenol 1 to 80	+	+	−	−	−	−

The experiments shown in Table XVIII indicate that the optimum amount of ammonia is approximately one-half of the molecular equivalent.

Experiments were next made with anthrax spores, using the following method: Equal quantities (2½ cc. each) of chlorin solution and spore suspension, with or without blood serum added to it, were mixed in a test tube and vigorously shaken. After it had stood at room temperature for the required time of exposure the mixture was again shaken, and a subculture was made by a standard platinum loop into a tube of nutrient broth. No attempt was made to neutralize any excess of disinfectant. The results of these experiments are shown in Table XIX.

TABLE XIX.—*Germicidal activity of chlorin against anthrax spores with and without addition of ammonia* [a]

EXPERIMENT 35

WITHOUT AMMONIA; 10 PER CENT BLOOD SERUM ADDED

Concentration of chlorin.	Exposed 1 hour.	Exposed 2 hours.	Exposed 3 hours.	Exposed 4 hours.	Exposed 5 hours.	Remarks.
1 to 1,000	+	+	+	+	−	The same dilutions without blood serum killed the spores in 30 minutes.
1 to 2,000	+	+	+	+	+	
1 to 4,000	+	+	+	+	+	

WITH ONE-HALF MOLECULAR EQUIVALENT OF AMMONIA; 10 PER CENT BLOOD SERUM ADDED

1 to 1,000	−	−	−	−	−	Number of spores 350,000, or 70,000 per cubic centimeter.
1 to 2,000	−	−	−	−	−	
1 to 4,000	+	+	−	+	−	

EXPERIMENT 36

WITHOUT AMMONIA; 10 PER CENT BLOOD SERUM ADDED

Concentration of chlorin.	Exposed 3 hours.	Exposed 6 hours.	Exposed 12 hours.	Exposed 18 hours.	Exposed 24 hours.	Remarks.
1 to 1,000	+	+	+	+	−	The same dilutions without blood serum killed spores in 15 minutes.
1 to 2,000	+	+	+	+	+	
1 to 3,000	+	+	+	+	+	
1 to 4,000	+	+	+	+	+	

WITH ONE-HALF MOLECULAR EQUIVALENT OF AMMONIA; 10 PER CENT BLOOD SERUM ADDED

1 to 2,000	−	−	−	−	−	Number of spores 350,000, or 70,000 per cubic centimeter.
1 to 4,000	−	−	−	−	−	
1 to 6,000	−	−	−	−	−	
1 to 8,000	+	+	+	+	+	

[a] + signifies growth; −, no growth.

The results shown in Table XIX seemed to show clearly that chlorin with ammonia added had very great germicidal value, even in the presence of organic matter in the form of blood serum.

Experiments were, therefore, undertaken to ascertain whether or not such a solution could be used for disinfecting hides. The technic was as follows: Small pieces of dry hide, cut to the same weight, were infected by soaking them in a suspension of anthrax spores and then drying them over sulphuric acid in a vacuum equal to about 5 mm. of mercury for 48 hours. These pieces of infected hide were then treated with the disinfectant solution in the proportion of 5 times as much solution as hide by weight. At the end of the required period of exposure the pieces of hide were transferred to a solution of sodium thiosulphate of sufficient strength to neutralize completely the disinfectant carried over by the hide. After neutralization the hair and more or

less of the hide surface were scraped off with sterile instruments and plated, using exactly neutral agar. The results of two such experiments are given in Table XX.

It should be noted in this connection that the stock solutions of chlorin water and Dakin's solution from which test dilutions were prepared by the machine devised by the Wallace & Tiernan Co., of New York, for the preparation of Dakin's solution, chlorin being run directly into water or a solution of sodium carbonate, as the case might be.

TABLE XX.—*Germicidal activity of chlorin water and Dakin's solution against anthrax spores on pieces of hide*

EXPERIMENT 37

CHLORIN WATER WITH MOLECULAR EQUIVALENT OF AMMONIA

Concentration of chlorin.	Exposed 2 hours.	Exposed 6 hours.	Exposed 12 hours.	Exposed 24 hours.
1 to 500	Plates overgrown.	Colonies too many to count.	16 colonies, 14 anthrax.	Many anthrax colonies.
1 to 1,000	do	do	Many anthrax colonies.	Do.
1 to 2,000	do	do	Plate overgrown.	Do.[a]

CHLORIN WATER WITH ONE-HALF MOLECULAR EQUIVALENT OF AMMONIA

1 to 500	Plates overgrown.	Colonies too many to count.	80 colonies, 70 anthrax.	20 anthrax colonies.
1 to 1,000	do	do	Plates overgrown.	40 anthrax colonies.
1 to 2,000	do	do	do	Colonies too many to count.

[a] No count possible on account of spreaders.

EXPERIMENT 38

DAKIN'S SOLUTION WITH NO AMMONIA ADDED

Concentration of available chlorin.	Exposed 6 hours.	Exposed 18 hours.	Exposed 24 hours.	Exposed 48 hours.
1 to 250	Plates all show heavy growth, spreaders plus discrete and confluent colonies of anthrax.			
1 to 500				

DAKIN'S SOLUTION WITH ONE-HALF MOLECULAR EQUIVALENT OF AMMONIA

1 to 250	1 colony[a]	10 colonies[a]	1 colony[a]	4 colonies.[a]
1 to 500	Many anthrax colonies.[b]	4 colonies, 2 anthrax.	No colonies visible.[c]	2 colonies.[a]

DAKIN'S SOLUTION WITH MOLECULAR EQUIVALENT OF AMMONIA

1 to 250	Many anthrax colonies.[b]	4 colonies	1 colony[a]	4 colonies.[a]
1 to 500	do	1 colony anthrax.	No colonies visible.[c]	1 colony.[a]

[a] Not anthrax. [b] No count possible on account of spreaders. [c] Covered by spreaders.

The results shown in Table XX indicate that the results previously obtained were too high, presumably because in the previous experiments there was no attempt to neutralize the disinfectant. However, on the addition of ammonia there is still evident a great increase in germicidal activity and much less decrease in germicidal power when organic matter is present.

A selective action upon the different types of organisms was also seen. Where the concentration of available chlorin was low and the time of exposure comparatively short the plates were overgrown by spreaders and various types of colonies, of which the anthrax colonies made a very small part. With greater concentration of chlorin and longer exposure this proportion was reversed, and most of the colonies seen were those of anthrax. In experiment 38 it was found that even after no anthrax colonies were to be found there were still spreaders and colonies of organisms other than anthrax. These organisms were not identified except to make sure they were not anthrax but were evidently already present on the hide and were more resistant than the strain of anthrax spores employed.

CONCLUSION

(1) In the ordinary routine work of general disinfection, such as disinfection of cattle cars and pens, there is always a large amount of organic matter present. It is evident, therefore, that because of the enormous diminution in germicidal value on addition of organic matter as well as because of the injurious effects on metals and fabrics the chlorin disinfectants as a class do not seem to be suited for use under the usual conditions and by the usual methods of general disinfection. That is not to say, however, that when properly used they are not efficient and valuable in the treatment of infected wounds; in fact, the evidence available goes to show that they are of great value when so used; and, of course, chlorin and hypochlorites are being very widely and successfully used for the disinfection of drinking water.

(2) Compared on a basis of weight of chloramin T as against weight of chlorin as sodium hypochlorite (Dakin's solution) or hypochlorous acid (eusol), or as chlorin in aqueous solution, chloramin T is less efficient than the others. But if the comparison is made on the basis of available chlorin contained it is much more efficient against *Staphylococcus aureus*, much less efficient against *Bacillus pyocyaneus*, and approximately equal in efficiency against *B. typhosus*.

(3) The experiments upon *Bacillus tuberculosis* indicate that the chlorin disinfectants are worth very little so far as that organism is concerned. This is not surprising in view of the use of antiformin ($NaOCl + NaOH$) in isolating tubercle bacilli.

(4) In the present work, considered as a whole, there is seen throughout more or less "selective action" on the part of the various disinfectants. The most clearly defined example of this is seen in the extremely high

value of chloramin T against *Staphylococcus aureus* as compared with its extremely low value against *Bacillus pyocyaneus*.

(5) The results of the experiments upon anthrax spores show that the germicidal action of chlorin compounds is not always so speedy as is commonly supposed but may extend over several days.

(6) The addition of ammonia to solutions of chlorin or hypochlorites very greatly increases germicidal activity and tends to prevent depreciation in value on the addition of organic matter.

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A NEW AVOCADO WEEVIL FROM THE CANAL ZONE

By H. F. DIETZ,[1] *Entomological Inspector*, with description of the species by H. S. BARBER, *Assistant, Bureau of Entomology, United States Department of Agriculture*

INTRODUCTION

The Federal quarantine against the avocado weevil (*Heilipus lauri* Boheman) led Mr. James Zetek, Entomologist of the Panama Canal, and the writer, during the service of the latter in the Canal Zone, to search for the weevil in the native avocados in Panama. The weevil proves to be a species previously unknown to science, but the results of investigations of the breeding habits of these potential pests, still very imperfectly understood, supply the first records of field observations under natural conditions.

Two closely related species of avocado weevils are known.[2] As the first, *H. lauri* Boheman, is indigenous to Mexico and the second, *H. pittieri* Barber, is native in Costa Rica, the existence of this new form had already been suspected.[3] Its discovery is of special interest, however, since it has been recently intercepted entering the United States.[4]

FIELD OBSERVATIONS

Two males of this weevil had been found in June, 1918, feeding on the leaves of small seedling avocado trees in a nursery at Ancon, C. Z., by Mr. Zetek, and further search was rewarded in April and May, 1919, when "wild" avocado fruits, the seeds of which contained Heilipus larvæ, were collected at the large avocado plantation at Frijoles, C. Z. These fruits came from large trees growing wild at the edge of a plantation and at a considerable distance from the cultivated, bearing trees. Attempts to determine the previous history of these "wild" trees were unavailing. Infested fruits were brought to the Board of Health Laboratory at Ancon, and the adults reared from them did not differ from the two collected in 1918 or from the large specimen which had been sent to the National Museum by Mr. F. H. Jackson about 1912. From the occurrence described above and from the date of the last-mentioned specimen it would appear that the species is endemic in Panama, but there remains a possibility that it

[1] Resigned Nov. 3, 1919.

[2] BARBER, H. S. AVOCADO SEED WEEVILS. *In* Proc. Ent. Soc. Wash., v. 21, no. 3, p. 53-60, pl. 2. 1919.

[3] A very large specimen was received at the United States National Museum about 1912 from Las Cascadas, C. Z. (F. H. Jackson, collector), but it was not treated in the paper by H. S. Barber cited above because of absence of data definitely associating it with avocado. Other close relatives with similar habits will undoubtedly be found in other avocado-growing regions of tropical America.

[4] This interception was made by Mr. O. K. Courtney, Port Inspector of the Federal Horticultural Board at New Orleans, La., in October, 1919. *H. perseae* Barber was found in an avocado seed in the baggage of a steamship passenger arriving at New Orleans from Cristobal, C. Z.

Journal of Agricultural Research,
Washington, D. C.
vd

Vol. XX, No. 2
Oct. 15, 1920
Key No. K-8

might have become established there long ago through the importation of avocados or their seeds from some other part of the American Tropics.

Miscellaneous information regarding the habits of the various stages of the weevil was obtained in rearing it. Some notes regarding its economic importance and distribution were also made.

At Frijoles only the "wild" fruits were infested, 17 out of 40, or 42.5 per cent, of such fruits containing from 1 to 4 larvæ. Out of over 200 cultivated fruits examined here not one was found infested. Fruits infested with Heilipus larvæ have been found on fruit stands in Panama City and Colon, in the Republic of Panama, and at Gatun and Ancon, in the Canal Zone. The only information obtainable in these cases regarding the origin of such fruits was that they came either from the Canal Zone or neighboring parts of the Republic of Panama. From the data at hand the species seems to be limited to the "Canal Zone region," though there is little doubt that it occurs over a much wider area.

EGG PUNCTURES AND LARVAL HABITS

The egg punctures are somewhat crescent shaped, about 4 mm. long, with the ends blunt. In a general way they resemble those of the plum curculio. As many as 10 were found on a single fruit, but in 8 of these the eggs had been crushed by the growing fruit and in 2 young larvæ had hatched. No eggs were found, but from the examinations of infested fruits it is evident that the eggs are laid at the junction of the skin of the fruit and the pulp. The exact time that oviposition takes place is not known, but from the evidence at hand it is when the fruit is between one-half and three-fourths mature.

After hatching, the larvæ often wander through the pulp before entering the seed, thus rendering a considerable part of the fruit inedible, especially where more than one larva occur in it. Once the larvæ enter a seed they confine their activities to it. Mr. Barber has called attention to the fact that seeds infested with *H. lauri* and *H. pittieri* do germinate if the embryo has not been injured by the tunnelling of the larvæ, and the same thing has been observed in the study of *H. perseae*; but when a seed becomes infested with two or more larvæ, it is usually so badly riddled that it can not germinate. Furthermore, seeds infested with Heilipus larvæ seem to be subject to the attacks of several kinds of "dry rots" which follow along the tunnels, invade the embryo, and kill it Likewise, these fungi, at least under laboratory conditions, seem to be indirectly responsible for the death of a considerable number of larvæ and pupæ.

No natural migration of larvæ from one seed to another, even when these seeds are massed together, has been observed, but half-grown larvæ taken from infested seeds immediately tunnelled into uninfested ones when these were provided.

The duration of the larval stage was not determined, but indications are that it is not less than three months.

PUPATION

When the larvæ are full grown, instead of leaving the seed they hollow out a large spherical cell in which they pupate. Three such cells have been found in one-half of a large avocado seed, and four adults have been reared from a single seed. This is probably as large a number of adults as can be obtained from one seed because of the quantity of food eaten by the larvæ and because of the fact that the larvæ tunnel freely from one cotyledon of the seed to the other. The minimum duration of the pupal stage is 12 to 15 days.

HABITS AND INJURY BY ADULTS

The adults, on transforming from the pupal stage, rest in the pupal cell from two to four days and then cut their way out. At the time they come from the pupal cell the adults are decidedly reddish in color, with six prominent yellowish spots, as given in the technical description. The reddish color becomes darker with age and is finally blackish in reared individuals that live over two months.

The adults readily drank water that collected on the sides of the glass cages to which they were confined. They ate and seemed to flourish on half-ripe fruit, young leaves, and stems of avocado and on fresh avocado seeds. In one case an individual that had been starved for a week ate a few holes in guava leaves.

Injury to the fruit and to the leaves and stems is shown in Plate 7, C, and in Plate 8. An interesting thing about the fruit injury is that the outer skin was first eaten off; then, as the surface of the pulp became dry a day or so later, this in turn was eaten off, the result being that within a week holes almost ½ inch deep were eaten out. On the young stems the bark layers were gnawed off first and the woody areas were then eaten through, so that all the parts above the injury collapsed. Similar injury was done to the petioles of the leaves. In inspection work at the Plant Inspection House of the Office of Foreign Seed and Plant Introduction, Bureau of Plant Industry, at Washington, D. C., avocado bud wood has repeatedly been received from Guatemala showing insect scarring similar to that caused by the light feeding of *H. perseae* on young stems. This injury on the Guatemala bud wood may have been the feeding injury of *H. pittieri* that had "healed over." In practically every way the feeding habits of *H. perseae* are similar to those of *H. lauri* as recorded by Barber.

The shortest time that any individuals of this new species (*H. perseae*) remained alive was 10 days, all of them without food. One male without food but with copious and regular supply of water remained alive 23 days. It was observed that when individuals were kept in dry cages they soon died, even in the presence of food. The longest time any individual remained alive was 116 days, this being a female.

Although five individuals (two males and three females) were kept together 35 days, no mating was observed, nor did oviposition take place on the half-ripe fruit that was provided for this purpose.

GENERATIONS

The apparently long duration of the larval stage and the known longevity of the adults indicate that there is but a single generation in a year. If this is true, then it is a long-drawn-out generation, for, from the material obtained at Frijoles, adults emerged over a period of 40 days, and in several cases a month elapsed between the emergence of the first and last adults from the same seed. It is probable, however, that breeding is controlled in the Tropics more by the activities of the host plants in supplying the proper conditions for oviposition.

CONTROL

The control of all three species of the genus Heilipus now definitely known to infest avocado seeds is comparatively simple, because pupation takes place inside the seed. It consists of gathering up and burning the fallen fruits and seeds. This control may be complicated, however, by the presence of "wild" trees that are not readily accessible or easily eliminated. In such cases it may be possible to protect cultivated fruits by arsenical sprays, for the adults feed freely on the leaves and doubtless in the field drink considerable water off the leaves when these are wet.

DESCRIPTION OF HEILIPUS PERSEAE

Heilipus perseae Barber, n. sp. (Pl. 7, A, B.)

Closely related to *H. lauri* Boh., but more robust; the squamose fascia of elytra larger, and, in addition, a similar squamose area on the sides of the pronotum. The rostrum is short in both sexes, and the mesosternum is not prominent. The legs are also much shorter than in either *H. lauri* or *H. pittieri*.

Ovate, shining, rufopiceous, clothed sparsely with scales which are white on legs and under surface, pale ochreous in the seriate elytral punctures, and darker ochreous on thoracic and elytral fasciæ, the marginal scales of which appear paler. Frontal fovea deep; eyes much larger than in *H. lauri* and separated above by less than half the width of rostrum; the latter shorter (eye to apex) than the pronotum in both sexes. Pronotum very coarsely sparsely punctate, median line impunctate but not elevated; lateral squamose areas irregularly oval, usually a little produced downward in the anterior constriction, but rarely extending to basal or apical margins. Scutellum small, subtriangular, convex, impunctate, polished. Elytra sparsely seriately foveolate, the foveæ densely squamose; two large squamose areas in same position as the small ones in *H. lauri*, the apical fascia usually extending from side margin to suture, but sometimes nearly divided at suture. Mesosternum a little produced but not projecting beyond coxæ. First and second ventral segments feebly impressed at middle in the female, a little more strongly impressed in the male. Tibial claws short and stout. Length (rostrum excluded) 11 to 15.5 mm., width 4.8 to 5.7 mm. Length of rostrum, males 2.9 to 3.4 mm., females 3.2 to 4.1 mm.

The sexes are extremely difficult to distinguish, unless the tip of the aedeagus or the "palps" of the ovipositor can be seen. Nine males and seven females are before me, all having been reared from avocado seeds at Frijoles, C. Z., by Mr. H. F. Dietz, during May, June, and July, 1919, except a male taken at Ancon, June 20, 1918 (J. Zetek No. Z1084), and a large undated female (the allotype) from Las Cascadas, C. Z., received from F. H. Jackson about 1912.

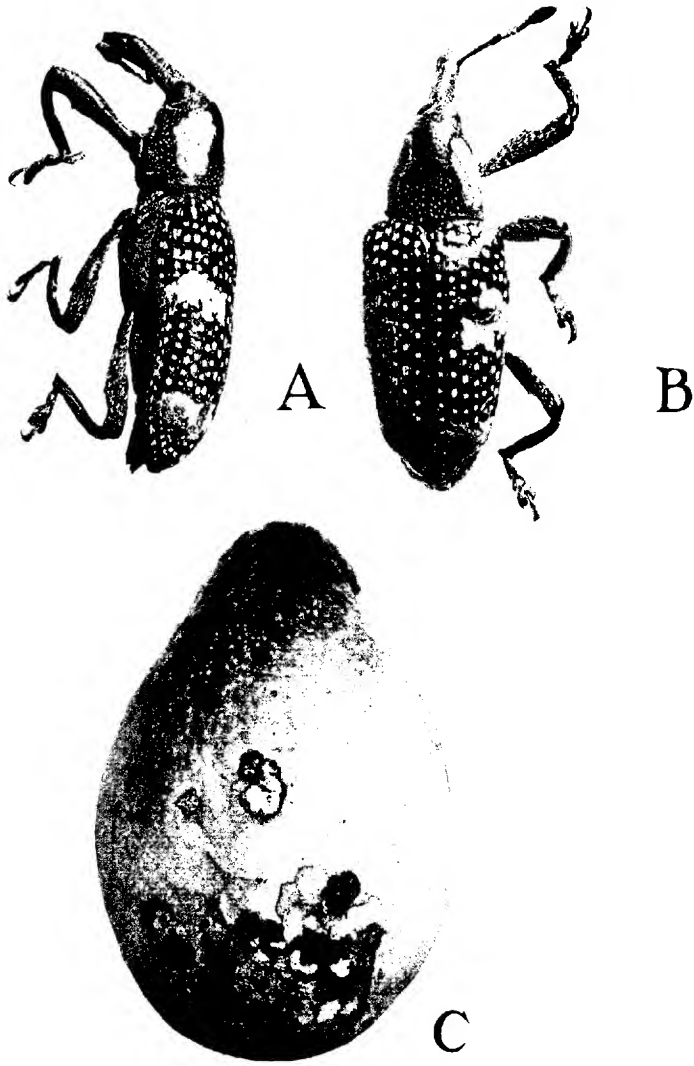
Type, allotype, and 14 paratypes, United States National Museum No. 22586. One paratype retained by Federal Horticultural Board and one paratype sent to Mr. Geo. C. Champion.

PLATE 7

Heilipus perseae

A, B.—Adult, paratype. × 5.

C.—An avocado fruit (reduced) showing feeding injury by the beetles.


A
B
C

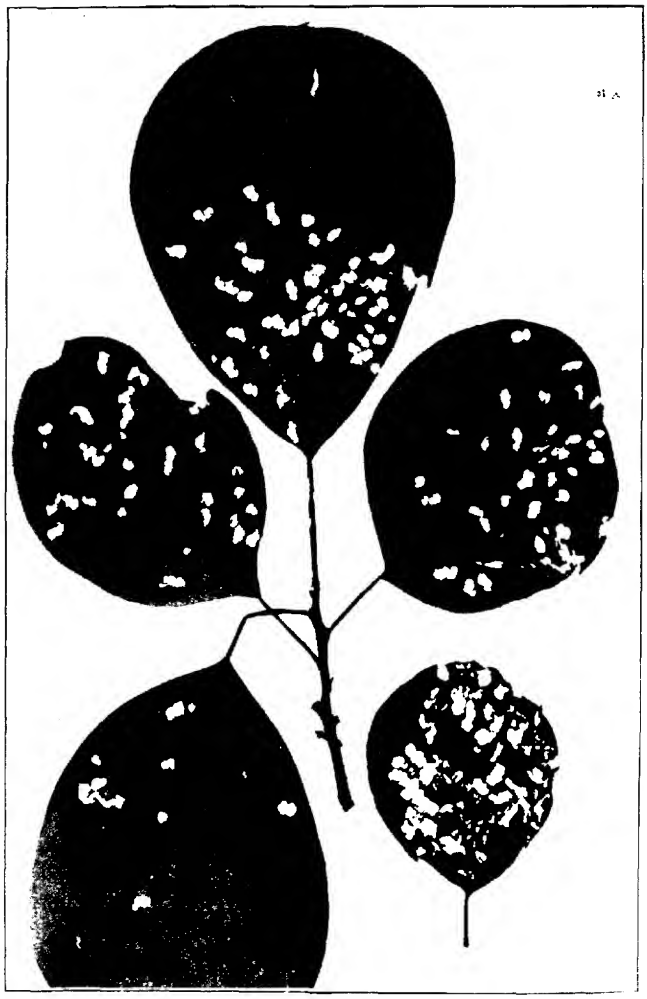


PLATE 8

Heilipus perseae:

Leaves showing the injury done by five beetles in 48 hours.

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PLATE 9

Heilipus perseae, mature larva:

A.—Ventral face of ventral mouth parts.

B.—Anterior part of head from above.

C.—Lingua, hypopharynx, hypopharyngeal bracon, and dorsal (buccal) face of maxilla.

D.—Dorsal face of mandible.

E.—Epipharynx.

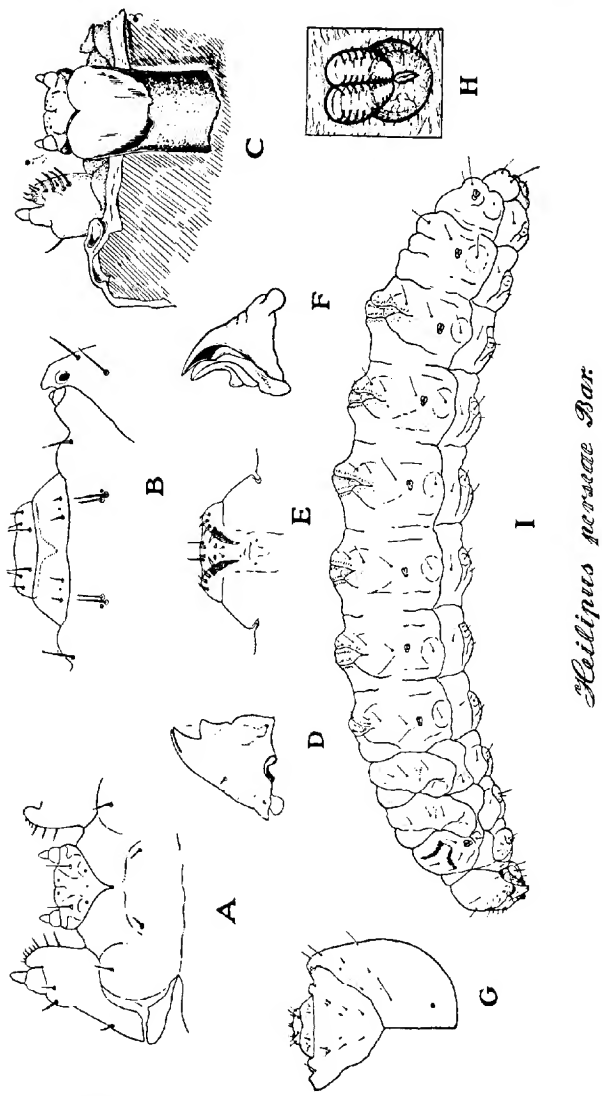
F.—Ventral face of mandible.

G.—Head capsule from above.

H.—Thoracic spiracle from outside.

I.—Mature larva.

Drawings, from studies, by Dr. A. G. Böving.


A
B
C
D
E
F
G
H
I
Heilipus perseae Bar.

STUDIES IN MUSTARD SEEDS AND SUBSTITUTES: I. CHINESE COLZA (BRASSICA CAMPESTRIS CHINOLEIFERA VIEHOEVER)

By ARNO VIEHOEVER, *Pharmacognosist in Charge*, JOSEPH F. CLEVENGER, *Assistant Plant Histologist*, and CLARE OLIN EWING, *Assistant Pharmacognosist, Pharmacognosy Laboratory, Bureau of Chemistry, United States Department of Agriculture*[1]

INTRODUCTION

Shortly after the outbreak of the recent great war many products which previously could be obtained from European countries were no longer available, and as a result importers were obliged to seek other sources of supply. One of the products thus affected was mustard seed. It was soon apparent that much of the seed offered for entry as mustard was quite different not only in quality but also in general appearance and condimental character from that which had usually been imported. Some of the shipments, for example, of Chinese mustard (*Brassica juncea* (L.) Cosson), while not so satisfactory as the mustards formerly recognized, consisted of seeds with condimental and medicinal qualities which made them useful as substitutes. Others, consisting of Japanese mustard (*41*)[2] (*Brassica cernua* Thunb.), proved to be very valuable material. It is probably grown under more favorable climatic conditions and is evidently collected more carefully than the Chinese seed.

Seeds from some other Brassica species which possessed no medicinal or satisfactory condimental value, however, were imported (*1, p. 469; 45; 46; 48*), and among these was the one to which this article has reference. The seed was first called to the attention of the authors because it had been imported in large quantities as rape seed and subsequently was introduced into interstate trade as mustard seed. Its appearance was rather bright, though not shiny, and resembled in a way yellow or white mustard (*Sinapis alba* L.) (*31, p. 379*). On account, however, of its peculiar earthy flavor and lack of the pungency characteristic of mustard, it did not meet with the unqualified approval of the trade.

[1] During the progress of the botanical work the authors obtained valuable assistance from the Bureau of Plant Industry, United States Department of Agriculture, and desire to acknowledge especially the help of Messrs. Brown and Hillman, of the Seed-Testing Laboratories; Mr. Shoemaker, of the Office of Horticultural and Pomological Investigations; Messrs. Fairchild, Bisset, Skeels, Stuntz, and Rankin, of the Office of Foreign Seed and Plant Introduction; Messrs. Coville and Blake, of the Office of Economic and Systematic Botany; and Messrs. Swingle and Tanaka, of the Office of Crop Physiology and Breeding Investigations. Prof. Trelease, of the University of Illinois, also kindly gave his advice. For valuable assistance in connection with the chemical work appreciation is due to Mr. Burnett, formerly of the Oil Fat, and Wax Laboratory; to Mr. Gowen, formerly of the Baltimore Food and Drug Inspection Station and especially to Mr. Bornmann, of the Chicago Food and Drug Inspection Station, all of the Bureau of Chemistry, United States Department of Agriculture.

[2] Reference is made by number (italic) to "Literature cited," p. 137-139.

CLASSIFICATION

IDENTIFICATION

While a preliminary study seemed sufficient to exclude the seed from the group of true mustards,[1] much difficulty was encountered in definitely identifying it. The material had evidently not been imported before, at least not in recent years, nor could similar authentic material be located in this country in any of the larger museums. Since the information on the subject in the literature was contradictory, insufficient, or entirely lacking, extended studies were undertaken to determine the macroscopic and microscopic characteristics of the seeds, as well as the chemical composition and certain physiological characteristics of the volatile oil. Plants were also grown to maturity, and the characteristics at the different stages of growth were determined. These experiments were correlated with data in the literature, as a result of which identification of the seeds as those of Chinese colza, *Brassica campestris chinoleifera*, n. var., was made possible. It should be mentioned here that Chinese colza was first classified by us (*1, p. 469; 45; 46*) as *Brassica campestris chinensis oleifera*, n. f. Upon suggestion of Messrs. Blake and Coville the name was changed to *Brassica campestris chinoleifera*, n. var., in order to avoid the use of a polynomial.

TAXONOMY

Some confusion exists concerning the nomenclature of Brassicas, the description of them in many instances being inadequate. This is especially true of the oriental species, of which the seed in question is a representative. Linnaeus (*26, p. 281*) described *Brassica chinensis* (Pl. 19, A) as a plant having stem-clasping leaves and slightly compressed siliques. It is obviously of the *Brassica campestris* type (Pl. 19, B).

Iinouma (*18*) described among other vegetables two plants which he called, respectively, Aburana (oil vegetable) and Tona (Chinese vegetable). Tanaka and Ono (*18*) identified Aburana as *Brassica chinensis* var., and Tona as *Brassica chinensis* L.

Ito and Matsumura (*19, p. 299–301*) include *Brassica chinensis* L. and *Brassica orientalis* Thunb. under the species *Brassica campestris* var. *chinensis* T. Ito. Kondo (*21, 22*) evidently accepted this classification and described Aburana, used for oil production, and (*4*) other forms, used for greens, as *Brassica campestris chinensis* T. Ito. Makino (*18*), apparently unaware of Ito's classification or Kondo's earlier work, identified both Aburana and Tona as *Brassica campestris* L. var. *chinensis* Makino. According to Georgeson (*16*), Abura-na, Nutum-na, and Chirimen-na[2] are Japanese names for Chinese cabbage, *Brassica chinensis* L.,

[1] Mustard seed is the ripe seed of *Sinapis alba* L. (white mustard), *Brassica nigra* (L.) Koch (black mustard), *Brassica juncea* (L.) Cosson, or the varieties or closely related species of the types of *Brassica nigra* and *Brassica juncea*, for example, *Brassica cernua* Thunb. (*42*).

[2] Free translation according to Georgeson: Na means green; abura, oil; nutum, rape seed; and chirimen, crape, referring to the crimped leaves of certain varieties.

which he, in agreement with Miquel (*29, p. 74–75*), considers identical with *Brassica orientalis* Thunb. He states (*16, p. 652*):

> No other vegetable of this class is so universally grown, or is represented by so many varieties. It is a kind of rape which has been transformed by cultivation. Certain varieties of it are grown only for their seed, from which an oil is expressed, formerly much used as lamp oil.

Judging by the illustrations and the very brief description given by these authors, and considering the great variations observed in plants grown from Chinese colza seed, it appears quite probable that both Aburana and Tona may be included in the series of plants treated as *Brassica campestris* var. *chinensis* by Lund and Kiaerskou.

Lund and Kiaerskou (*28, p. 166–167*), who carried on extensive growing and crossing experiments, classify under the name *Brassica campestris* var. *sativa annua chinensis* two forms of Chinese vegetables, Pe-tsai and Pak-choi.

Prain (*32, p. 42, 45*) gave to Pak-choi (Chinese cabbage), which he found growing on the Indian plains, the name *Brassica chinensis* L., including in this species also the plants described under the following names: *B. chinensis* L. var.; *B. campestris* Forbes and Hensl. in part, not of L.; *B. juncea* Forbes and Hensl. in part, not of H. f. et. Th.; *B. oleracea* L. var. *chinensis* Prain; *Sinapis brassicata* L.; *Pak-choi* Vilmorin; *Pak-tsoi* Roxb.; *Yea-tsoi* Roxb.

Vilmorin (*49, p. 491*) classifies under *Brassica chinensis* L., in addition to Pakchoi and Pe-tsai, a third form of less cultural interest which has almost entire leaves with narrow petioles.

Stuart (*38, p. 73*) classifies only Pe-tsai or Pai-tsai under *Brassica chinensis* and states that it is a most common variety of *Brassica oleracea*. He points out, however, that Yu-tsai,[1] undoubtedly *Brassica rapa*, yielding rape seed from which rape-seed oil is manufactured, is also called *Brassica chinensis*, possibly on account of its economic prominence in China.

Bailey (*3*) refers to Pak-choi and Pe-tsai as two different species, calling the first *Brassica chinensis* L., and the latter *Brassica pe-tsai*. He considers that Linnacus' description for *Brassica chinensis* answers best for Pak-choi.

Gagnepain (*15*) has renamed Pe-tsai, classified by Loureiro (*27, p. 400*) as *Sinapis pekinensis*, *Brassica pekinensis* (Lour.) Gagnepain. Skeels (*43, p. 21*), evidently unaware of Gagnepain's classification, renamed the same form *Brassica pekinensis* (Lour.) Skeels.

Duthie and Fuller (*10, p. 33–34*) give the name *Brassica chinensis* to a plant with many characteristics of *Brassica juncea*, but they point out that they consider *Brassica chinensis* Duthie and Fuller synonymous with *Sinapis chinensis* L. The choice of the name *Brassica chinensis* is unfortunate for a plant with characteristics of *Sinapis chinensis* L. and apparently identical with or closely related to *Brassica juncea* (L.) Cosson.

[1] Dr. Yamei Kin, familiar with China and its agricultural products, suggested that the material which the authors considered as Chinese colza was Yu-tsai. However, since the seeds examined by the authors differed from samples obtained as Yu-tsai from China, it appears that this name is not definite.

TERMINOLOGY

SCIENTIFIC NAME

While it is believed that the plants grown from material in the Pharmacognosy Laboratory (Pl. 18, A), show characteristics typical of the plant described by Linnaeus as *Brassica chinensis*, and while they apparently agree rather well, so far as the general morphology of the plant is concerned, with Pak-choi (Pl. 15, C), there are certain differences, especially in the seeds, from Pak-choi as well as Pe-tsai. The seeds of Pak-choi and Pe-tsai were generally found to be smaller, more spherical, and usually of a brown color. As a rule, they show even less marked reticulations than the brown seeds of the authors' material. The most striking differences observed in the plants is the lack of broad petioles (see also Vilmorin's description of one form) and the failure to form heads in the rosette stage, so strongly developed in Pak-choi and especially in Pe-tsai (Pl. 15, A). These differences, however, while distinct, are not so marked that they might not be considered to fall within the latitude of species character. It would, therefore, seem that the laboratory material might be classified as a variety of the type species *Brassica chinensis* L. were it not for the following reasons. The description which Linnaeus gives is very brief, in fact so brief that much of the confusion in the use of this specific name by different authors is probably due to this limited species description. Bailey points this out, giving still another instance where the name *Brassica chinensis* has been used, evidently not correctly (*3, p. 543*):

> It is impossible to determine whether this particular plant [Pak-choi cabbage] is the one that Linnaeus meant to distinguish by his *Brassica chinensis*, but it best answers the description in his Amoenitates (Vol. IV). In Linnaeus's herbarium is a Brassica marked "chinensis" in his own handwriting, but it shows purple fls. and has lyrate-lobed lvs., whereas Linnaeus described his plant as having yellow fls. and cynoglossum-like lvs., probably not the original.

Linnaeus's description, nevertheless, indicates the close relation to *Brassica campestris*, and Lund and Kiaerskou showed this close relation by classifying both Pak-choi and Pe-tsai as *Brassica campestris* var. *annua sativa chinensis*.

Bailey (*2, p. 188*) takes a different stand:

> In common with all members of the genus Brassica, or cabbage and mustard tribe, these Chinese plants are much confused respecting their botanical characters. Recent writers have referred all the Chinese cabbages to *Brassica campestris*, the rutabaga; but one who studies the plants carefully, both from herbarium and living specimens, can not long hold this opinion. The genus Brassica divides itself naturally into two groups—the cabbages and rape, characterized by thick leaves, very glaucous-blue herbage and long flowers which are creamy white, and the mustards, with thinner and green or lightly glaucous herbage and small, bright yellow flowers. The Chinese cabbages belong to this latter group rather than to the former. Their flowers are those of the mustards, and I have no hesitation in removing the plants from *Brassica campestris*.

He thus takes a different stand from all other botanists who have given attention to these "Chinese cabbages and mustards." The authors of this paper also disagree with Bailey's viewpoint and classification on the basis of a rather extended investigation reported in the following paragraphs. There is no doubt in their minds that the so-called Chinese cabbages are not mustards but belong to the colza group, *Brassica campestris* L.

Concerning *Brassica campestris* L., Prain (*32*) states:

From the standpoint of commerce it is a matter of supreme indifference whether campestris, napus, and rapa be treated as separate species or subspecies of one and the same species.

Consequently, in his systematic synopsis he proposes a number of groups:

(1) *Brassica oleracea*, cabbage group; and (2) *Brassica campestris* Linn. ampl. Subspecies A, *campestris* (sp. Linn.), representing the colza group, subspecies B, *napus* (sp. L.) representing the rape group, and subspecies C, *rapa* (sp. L.) representing the turnip group.

As to the close relationship of the respective forms, Bailey states (*3*, *p. 544*) that he—

ound no difficulty in crossing cabbage-kale-cauliflower and others.

Lund and Kiaerskou, especially, showed by extensive crossing experiments the close relationship of *Brassica oleracea*, *Brassica campestris*, and *Brassica napus*. Notwithstanding this close relationship, however, it appears necessary to go further than Lund and Kiaerskou (28) in the classification of some of these forms, for instance in the classification of Pak-choi and Pe-tsai. Bailey states (*2*, *p. 189*)—

there is even good reason for separating the two types of Chinese cabbage . . . into two species, for they differ widely in their leaf characters and pods; and the former [*Brassica pe-tsai*] is truly annual, while the latter [*Brassica chinensis*] is evidently normally biennial.

Although the authors did not study these forms extensively, Shoemaker has shown that they can be readily crossed (Pl. 15, B) and therefore should not be considered as having species character (*4*). It is at present impossible to state definitely the relationship of Chinese colza to these forms. Since it has greater similarity to Pak-choi than to Pe-tsai, it appears not unlikely that Chinese colza and Pak-choi have developed from one common stock. Pe-tsai may present a further modification of Pak-choi, since, it is said, plants with narrower petioles may develop from Pe-tsai seeds. Pending further collection of data on Pe-tsai and Pak-choi the following classification, based on that of Bailey, Lund and Kiaerskou, Gagnepain, and others, appears satisfactory for the separation and identification of these horicultural and oil-yielding forms:

1. Pak-choi, *Brassica campestris chinensis* T. Ito.
2. Pe-tsai, *Brassica campestris pekinensis* (Lour.) Viehoever.
3. Chinese colza, *Brassica campestris chinoleifera* Viehoever.

This classification appears the more satisfactory, at least so far as Chinese colza is concerned, since it indicates clearly the very close relationship to Indian colza, *Brassica campestris* var. *glauca* Watt. This relationship is evident from botanical characteristics of the plant, and especially from the morphological and anatomical characters, as well as from chemical characters of the seeds of both Chinese and Indian colza.

POPULAR NAME

The popular name "Chinese colza" has been selected on the basis of the findings enumerated. Furthermore, it appears preferable to "China" or "Chinese cabbage," names often used for similar seeds, especially for Pe-tsai or other related horticultural varieties. Tracy (*39, p. 603*) states:

> The Chinese cabbage of this country is a wholly different species from the common cabbages. Chinese cabbage does not form a compact and rounded head. . . .

Georgeson (*16, p. 652*) states:

> The term cabbage is a misnomer, as its resemblance to that vegetable is quite remote. The plants are merely bunches of large, smooth, more or less spreading leaves, with broad fleshy midribs. They do not bear their leaves on a well defined stem, as do the cabbage, the kale, etc., but look more like the Cos lettuce, the leaves having their origin at the surface of the ground.

Learning also that certain forms of *Brassica oleracea*, apparently peculiar to China, are grown there, the authors felt that the name "Chinese cabbage" could properly be applied only to those.

The authors' form, although rather closely related to *Brassica oleracea*, is primarily an oil-yielding form which does not head and which deserves the designation "cabbage" even less than Pe-tsai and Pak-choi, both more or less heading forms. Some consideration was given the name "Chinese yellow rape," as the seeds resemble rape seeds in a way and yield a fixed oil similar to rape oils. In order to avoid confusion in horticultural nomenclature and to protect the agriculturist, however, it was considered advisable to adopt the more specific name of "Chinese colza."

BOTANICAL STUDIES

DESCRIPTION OF SEEDS

The seeds (Pl. 10, A, B) of Chinese colza, *Brassica campestris chinoleifera* Viehoever, are yellow or brown, and, if immature, green in color. In mass they have a dull yellow color, due to the preponderance of yellow seeds. In form they are somewhat compressed, oval, and usually with distinct ridges on one side. The size varies from 1.4 to 2.6 mm. in the long axis. The weight varies from 1.4 to 6.4 mgm., with an average weight (based on 1,000 seeds) of 2.865 mgm. The weight of 500 mils (quantity filling a 500-mil measure cylinder to the 500-mil mark) was 352 gm.

The surface usually appears smooth (Pl. 10, A, B) but under a hand lens shows very weak reticulations on the yellow seed and more distinct, but by no means prominent, reticulations on the brown seed (Pl. 10, C, D).

In cross section under the microscope the epidermis of the seed coat (Pl. 10, E, F, *a*) is striated tangentially, does not show any cell structure as in the mustard seed, and is about 5 microns thick. It does not swell appreciably when moistened and does not show crosses with polarized light. The sclerenchymatic palisade cells (Pl. 10, E, F, *c*) vary more in height in the brown seed than in the yellow. This explains the presence of the more pronounced reticulations in the brown seeds. For the yellow seed the height is almost uniformly 20 microns, while the average for the brown seed is about 25 microns, with a maximum height of 31 microns. The limits found for all the seeds were 15 to 31 microns high by 8 to 15 microns wide. The cell walls are strongly thickened at the base and sides, and the inner walls are smooth. The lumen contains no color substance. The parenchyma, always developed to one or more rows in the *Brassicas* (*40, p. 615*), is compressed to such an extent that it appears to be almost entirely lacking (Pl. 10, E, F, *b*). In *Brassica nigra* one row and in *Sinapis alba* two rows of parenchymal cells are clearly visible, even in the mature seeds.

The parenchyma (Pl. 10, E, F, *d*), located below the palisade cells, consists mainly of one row of cells which in the yellow seeds contain no color substance but in the brown seeds are filled with pigment. The endosperm (*e*) is characterized by the protein layer, a row of cells usually one cell wide, but occasionally two cells wide, the cells varying in height from 15 to 21 microns and in width from 15 to 42 microns and containing protein masses. The tissue (*f*) located below this layer is composed of several layers of parenchyma cells which, especially in the mature seeds, are strongly compressed. The embryo consists of two cotyledons folded in a characteristic way around the radical. The tissue is parenchymatic or meristematic. The cells which form the cotyledon tissues are not characteristic except that they contain globules of fatty oil, protein masses, and, especially in the immature state, a limited number of small starch grains which range in size up to about 6 microns in diameter. Experiments to locate the glucoside as a crystalline body have been unsuccessful. Studies to locate the enzym and glucoside microchemically in the cells are being undertaken.

DESCRIPTION OF THE PLANT

Experiments in the growth of selected yellow and brown seeds were made under greenhouse and field conditions. The field experiments were made at Arlington, Va., during the summer of 1916, and at Yarrow, Md., during the summer of 1917. The laboratory records, so far as differences in stages of growth are concerned, are more complete for the plants grown in the greenhouse.

The plants in all stages of their growth were generally smooth, with entire leaves. The young leaves, however, especially if grown in humid atmosphere, were more or less hairy, mainly on the margins (Pl. 11). In older leaves hairs were observed only occasionally. It was noted that isolated plants showed variations in the lobing, the leaves in some instances being deeply notched (Pl. 13). Experiments are being carried on to determine the latitude and significance of these variations. The appearance of some of these lobed leaves was very similar to that described for *Brassica napiformis* Bailey (*Sinapis juncea* var. *napiformis* Paill. and Bois), an observation which has much significance in view of Bailey's statement (3) that—

> it is nearly related to pak-choi, and it may have sprung from the same species; but it is clearly distinguished by its sharply toothed lvs. . . .

In the early stages the cotyledons had the same general appearance but were somewhat larger and thicker than those of the following mustards, *Brassica nigra* (L.) Koch, *Brassica juncea* (L.) Cosson, and *Brassica cernua* Thunb.[1] They were about 1 cm. long and 1 cm. broad, exclusive of the petiole, and are heart-shaped and smooth (Pl. 11). The first leaves were obovate, variously toothed, and somewhat crenate, and were hairy, especially on the margin if the seedling had been grown in very humid atmosphere. The leaves had a long petiole and a mid vein extending at least one-third of the length of the blade (Pl. 11).

In the late rosette stage (Pl. 14, 15) the leaves were arranged in a loose cluster, the wings of the leaf extending along the greater portion of the petiole, with the margin of the leaf more or less wavy and almost entire. The time required for the development of the full-grown rosette stage varies with the conditions for growth, being on the average about two months when grown under normal conditions in the field and about three months in the greenhouse. This period is materially shortened when there are conditions decidedly unfavorable for growth, such as insufficient nutriment, insufficient moisture, or too high temperature.

The early flowering stage (Pl. 16, 17) is characterized by a few erect branches up to 1 foot in length. The early stem leaves are similar to the rosette leaves, being almost entire, and obovate with long petioles. The upper stem leaves are variously stem-clasping, entire, somewhat glaucous and somewhat lanceolate acuminate. Many of the leaves of the secondary stems are not stem-clasping. The mature plant reaches a height of about 2 or 2½ feet, branching, and often showing an enlarged stem base (Pl. 16, A).

The flowers (Pl. 17, B), which are somewhat larger than those of *Brassica nigra*, *B. juncea*, and *B. cernua*, are in dense wide corymbs, 1½ inches

[1] Plants of *Sinapis alba* need not be considered in the comparison, since they are distinctly different from the other forms and can readily be recognized by such characters as the abundance of typical hairs on the entire young plant, as well as on the later plants, especially the pods, which themselves are readily distinguished by their typical shape.

long and 2 inches across when the flowers are open, subsequently elongating into racemes 6 to 18 inches long, with pedicels ½ to 2½ inches long in the extreme, slender, and without bracts or bractlets. The long pedicel particularly distinguishes the flower from the flowers of the mustards, which rarely have pedicels longer than ½ inch (*17*). Otherwise the flowers do not differ essentially from the general type of the genus Brassica.

The mature fruit pods (Pl. 18, B) are 2-valved, and are 2 to 3 inches long, including the beak. The beak of the pod is rather thickly conical and from 0.4 to 0.8 inch long. The valves are convex, rigidly leathery, rather finely nerved, and beaded opposite the seeds. A cross section of the pod is broadly elliptical throughout the entire length and about ¼ inch thick across the long axis. In some of the pods both yellow and brown seeds have been observed, giving evidence that the yellow and brown seeds are only variations in the same kind of seed. An examination of plants grown from brown and yellow seed will also prove this statement to be correct (Pl. 12, A). The green seeds are immature, as is indicated by the abundance of small spherical starch grains occurring in the cotyledons. From 8 to 12 seeds are found under each valve of a fully developed fruit pod.

BOTANICAL CONCLUSIONS

On the basis of the descriptive data given, the authors' material must be classified with the colzas and rapes rather than with the true mustards. While some of the characteristics observed would have only a limited diagnostic value if taken alone, they serve as additional means for the differentiation. Considered together, they make the proper classification the more certain. The botanical characteristics may be briefly recapitulated as follows.

SEEDS

1. As is typical of the colza group, the seeds are rather smooth. True mustards, except *Sinapis alba* L., show generally a more pronounced reticulation of the seed coat.

2. As in the case of Indian colza (*Brassica campestris* var. *glauca*), the seeds are more or less flat. True mustards are generally spherical, except *Brassica besseriana* Andrews, which has large brown seeds of more or less oval shape. Many rapes and Brassicas other than mustard, however, are also spherical.

3. A very pronounced ridge can be found in almost every seed of the Chinese and Indian colza, while it is scarcely developed in the mustard seeds, with the possible exception of *Sinapis alba*.

4. The swelling and polarizing epidermis is lacking in the Chinese colza seed, as usually also in other seeds of the colza group. While not so distinct or appreciable in certain forms or variaties of *Brassica juncea*, the swelling of the mucilaginous epidermis and the polarization are

especially pronounced in *Brassica nigra*, *Brassica besseriana*, and *Sinapis alba*. Swelling, however, has been observed in cabbage seed, *Brassica oleracea bullata gemmifera* (*40, p. 615 and table*).

5. The form and size of the palisade cells of the seed coat are similar to those of the general type found in the colza group and differ more or less strikingly from the true mustards.[1]

PLANTS

1. The tendency to rosette-like growth of plants in the early foliage stage, great in plants belonging to the colza group, was also observed in the authors' material. With the exception of certain variations of *Brassica juncea*, the authors have not observed a similar tendency in mustard plants.

2. The almost entire lack of hairs, especially pronounced in more advanced plants, has been noted on the plants studied, as well as on other plants of the colza group, a possible exception being *Brassica rapa*, reported by Bailey. In contrast, the plants of mustards are more or less distinctly hairy.

3. The upper leaves of the flower stalk are stem-clasping, as is general in the colza group; no distinctly stem-clasping leaves have been observed in plants of true mustards.

4. The pedicels (stalks of the flowers) of Chinese, as well as those of other colzas, average well over ½ inch in length, while those of the mustard flowers average less than ½ inch.

5. The greater length of the pods of Chinese and other colzas, often more than 2 inches, including the beak, frequently distinguishes them from the mustards, which, as a rule, have shorter pods, averaging usually less than 2 inches. Bailey (*2*), however, reports short pods for Pe-tsai.[2]

CHEMICAL STUDIES

GENERAL COMPOSITION OF SEEDS

The chemical studies included the general composition of a number of samples of the seed, as well as a more detailed examination of the fixed and volatile oils. Table I shows the composition of typical samples of the seed.

Judging from the composition of the seed and the low amount and character of the volatile oil yielded, the authors believe that the pressed oil cake will be a very good feeding material.

[1] For further details and comparison with other cruciferous seeds, the key given in Winton (*51, p. 173–189*) may be consulted.

[2] For further information and comparison, see Bailey (*3*), Howard et al. (*17*), and textbooks on taxonomy.

TABLE I.—*Analyses of seeds of Chinese colza (Brassica campestris chinoleifera Viehoever)*[1]

Sample No.	Moisture.	Ash.	Ether extract.[2]	Protein (N×6.25).	Reducing substances as starch by acid hydrolysis.	Crude fiber.	Volatile oil (crotonyl isothiocyanate).[3]	Iodin No. on ether extract (Hanus).
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
1	4.12	8.64	39.89	22.76	11.41	3.94	0.43	.....
	4.11	8.62	40.22	22.76	11.34	3.86	.43	.....
2	4.07	5.51	42.19	24.08	11.26	3.83	.57	99.8
	4.07	5.45	42.10	24.78	11.29	3.93	.54	99.8
	.....	.....	.....	.....	.....	.....	.....	99.4
3	4.07	8.45	40.25	22.72	11.64	3.99	.49	99.6
	4.04	8.49	40.30	22.85	11.71	4.33	.50	.....
4	3.86	8.41	40.35	22.89	11.70	3.96	.47	98.6
	3.88	8.39	40.42	22.76	11.65	4.03	.48	.....
5	5.25	5.14	42.4	24.38	.....	4.09	.52	.....

[1] Analyses of samples 1 to 4 were made by J. H. Bornmann, of the Chicago Food and Drug Inspection Station, Bureau of Chemistry, United States Department of Agriculture. Analysis of sample 5 was made by P. L. Gowen, formerly of the Baltimore Food and Drug Inspection Station, Bureau of Chemistry.

[2] Determinations of ether extract on two other samples, made by L. B. Burnett, formerly of the Oil, Fat, and Wax Laboratory, Bureau of Chemistry, showed 48.65 and 51.40 per cent, respectively.

[3] Analyses of samples 1 to 4 were made by the method of Vuillemin (*50*); analysis of sample 5 was made by the method outlined in this paper (p. 128); other determinations were made by the method given in the Official Methods of the Association of Official Agricultural Chemists. WILEY, H. W., ed. OFFICIAL AND PROVISIONAL METHODS OF ANALYSIS, ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS. As compiled by the committee on revision of methods. U. S. Dept. Agr. Bur. Chem. Bul. 107 (rev.), 272 p., 13 fig. 1908. Reprinted in 1912.

ISOLATION AND IDENTIFICATION OF VOLATILE OIL

A chemical investigation of the volatile oil was made in order to determine whether it should be properly classified with the volatile oil obtained from rape seed or with that of true mustard. The following procedure was employed to isolate the volatile oil:

Two kgm. of the seed in the form of a No. 20 powder were placed in an 8-liter flask; 4 kgm. of water were added; and the mixture was allowed to macerate for two hours at about 37° C. The mixture was then distilled with steam, and the distillate was saturated with salt and extracted with ether. The ethereal solution was dried over anhydrous sodium sulphate, and the greater part of the ether was distilled off, the last portions being allowed to evaporate spontaneously.

The volatile oil thus obtained had a specific gravity of 0.960 at 25°/25° C., and the distilled oil had a boiling point of between 165° and 172° C. (uncorrected) at 754 mm. These findings agree fairly well with those for crotonyl isothiocyanate, the volatile oil previously reported in rape by Sjollema (*35*, *36*). The thiourea and phenylthiourea derivatives were prepared, and their melting points and nitrogen content were determined. The results are shown in Table II.

TABLE II.—*Physical constants of allyl and crotonyl isothiocyanate*

Substance.	Specific gravity.	Boiling point.	Thiourea.		Phenylthiourea.	
			Melting point.	Nitrogen.	Melting point.	Nitrogen.
		°C.	°C.	*Per cent.*	°C.	*Per cent.*
Allyl isothiocyanate	1.013 to[1] 1.020 (25°/25° C.)	[1] 148 to 154	74	[2] 24.12	[3] 98.5	[3] 14.74
Crotonyl isothiocyanate	[2] .9933 (11°/4° C.)	[2] 174 (approximate).	[2] 64	[2] 21.25	[3] 53	[4] 13.00
Oil in question	.960 (25°/25° C.)	165 to 170	64	20.74	54 to 55	13.20

[1] U. S. P. IX (1916). [2] Sjollema (1901). [3] Stein (1907).

From these data it may be seen that the oil consists largely of crotonyl isothiocyanate, which, since it is the chief constituent of volatile oil of rape, corroborates the botanical findings that the seed is related to the rapes and not to the mustards. It was noted that the crotonyl isothiocyanate did not have the odor of volatile mustard oil (allyl isothiocyanate) but had an odor suggestive of turnip or cabbage. Furthermore, it did not have the typical irritating effect of mustard oil on the mucous membrane of the nose and on the eyes nor a blistering effect on the skin.

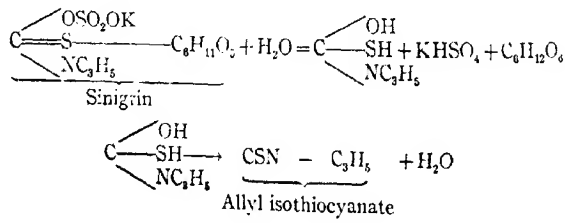
DETERMINATION OF VOLATILE OIL IN MUSTARD SEED AND MUSTARD SUBSTITUTES

In the course of this work it became necessary to determine the amount of volatile oil yielded by different varieties of mustards and mustard substitutes. Reference to the literature showed that there had been marked variation in the methods followed by different analysts in the determination of volatile mustard oils, especially in regard to the time of maceration and the conditions for distillation. Wehrmann, Wegener, Braunwarth, and Meyer (*51*) made an extended study of a number of these methods in order to arrive at a quick, convenient method for the determination of the volatile oil. In general, the studies here reported have corroborated their findings, except with respect to the effect of alcohol added before maceration (*51, p. 325*). Carles (*7, 8*) has also contributed valuable data to the solution of this problem. As a result of these studies, the following method, based largely upon that of Gadamer (*13, 14*), is recommended.

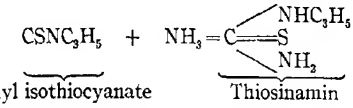
METHOD

Place 5 gm. of the ground seed (No. 20 powder) in a 200-mil flask, add 100 mils of water, stopper tightly, and macerate for 2 hours at about 37° C. Then add 20 mils of U. S. P. alcohol (95 per cent), and distill about 70 mils into a 100-mil volumetric flask containing 10 mils of 10 per cent ammonium-hydroxid solution and 10 mils of *N/10* silver nitrate solution. Mix thoroughly, stopper, and set the distillate aside overnight, heat to boiling on a water bath (in order to agglomerate the precipitate), cool, make up to 100 mils with water, and filter, rejecting the first portions. Acidify 50 mils of the filtrate with about 5 mils of concentrated nitric acid and titrate with *N/10* ammonium thiocyanate, using 2 mils of 10 per cent ferric-ammonium-sulphate solution for an indicator. Each mil of *N/10* silver nitrate consumed is equivalent to 0.004956 gm. of allyl isothiocyanate or 0.005657 gm. of crotonyl isothiocyanate.

The method is based on the hydrolysis of the glucoside by an enzym, both present in the seed. A volatile oil, glucose, and potassium hydrogen sulphate are formed.

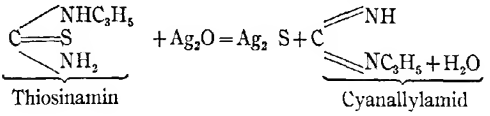


The volatile oil, readily volatile with the alcohol and water vapor, reacts with ammonia and silver nitrate. In the case of allyl isothiocyanate (the true volatile mustard oil) mainly allyl thiourea (thiosinamin) is first formed.



Allyl isothiocyanate — Thiosinamin

This reacts slowly in the cold but is completely decomposed by heating with silver nitrate, silver sulphid and cyanallylamid being formed.



Thiosinamin — Cyanallylamid

Both are insoluble compounds. They are filtered off, and the silver not used up in the reaction is determined volumetrically after Volhard.

1 atom of silver = ½ molecule of the volatile mustard oil.

Other compounds may also be formed in small amounts during the process (*13*, *25*).

NOTES ON METHOD

Carles (7) suggests a smaller sample, 3 or 4 gm., in the case of partially defatted samples or others yielding especially large amounts of volatile oil.

The seed used for analysis should, if possible, be freshly ground, as the powdered material loses its strength through hydrolysis, especially if not kept thoroughly dry—at or below 7 per cent moisture, according to Carles, not exceeding 2 per cent according to Boutron (5).

Joergensen (*20*, *p. 9*) and van Kampen (*44*, *p. 63*) in testing rape-seed cakes recommended the addition of thymol; Brioux (*6*, *p. 262-263*) recommended the addition of sodium fluorid to the rape-seed cake when this is macerated and tested for the amount of volatile oil available. They found that bacterial action would thus be largely inhibited in the maceration and higher yields would be obtained. Brioux used 2 gm. of sodium fluorid for 25 gm. of cake and 500 mils of water; van Kampen used 10 mils of 1 per cent alcoholic thymol solution added to either 25 gm. of cake or 5 gm. of mustard seed and 300 mils of water. Joergensen used a 1 per cent alcoholic solution of thymol and also in other experiments mercuric chlorid, which, however, proved unsatisfactory. Raquet (*33*) macerated the material in an aqueous alcoholic solution, adding 15 cc. of alcohol to the mixture before and 5 cc. after maceration, thus obtaining seemingly higher yields. We (*47*) could verify his findings but are still undecided whether this higher result is due to the formation of other volatile reducing substances or, as Raquet claims, to the fact

that in the presence of alcohol during maceration no bacterial fermentation causing a loss of volatile oil takes place.

A glycerin bath may be used to secure greater uniformity in heating.

The use of ground glass joints in the distilling apparatus has been recommended in literature in order to avoid possible errors caused by the use of rubber stoppers.

To insure complete absorption of the volatile oil, the tip of the condenser should always dip below the surface of the liquid in the receiving flask. It is advantageous to have the condenser terminate in a tube of small bore. A second receiver, containing ammonia and silver solution, may be used in case the completion of the distillation is in doubt.

According to Kuntze (*25*), the mixture obtained after distillation may also be heated directly without standing to 100° C. for an hour, using a reflux condenser or a long glass tube as a condenser. Possible further formation of an urethane compound (allyl urethane in the case of allyl mustard oil) can thus be avoided.

YIELD OF VOLATILE OIL

Examination by the method just given of a number of samples of seeds obtained from Chinese colza showed that the content of crotonyl isothiocyanate varies from about 0.4 to 0.6 per cent. Various attempts were made to increase the yield of volatile oil by addition of different chemicals before maceration. Table III shows the results of these experiments.

TABLE III.—*Effects of chemicals added before maceration upon the yield of volatile oil*

Substance added to 5 gm. of sample.	Volatile oil (crotonyl isothiocyanate).
	Per cent.
Potassium hydroxid, 0.2 gm	0.08
	.11
Potassium fluorid, 0.2 gm	.41
	.54
Tartaric acid, 0.2 gm	.30
	.42
Tartaric acid, 5 gm	.05
	.05
Tartaric acid, reflux 2 hours, 5 gm	.05
	.05
Sinapis alba, as a source of myrosin, 5 gm	.52
Alcohol, 20 cc	.79
	.79
No chemical	.55
	.58

The results shown in Table III may be summarized as follows: Five gm. of tartaric acid probably destroyed the enzym and no appreciable yield of volatile oil was obtained; 0.2 gm. of tartaric acid slightly retarded

the reaction. Water alone gave results averaging about 0.6 per cent of crotonyl isothiocyanate. The addition of 20 cc. of alcohol before the maceration gave a higher percentage of volatile oil, the results reaching almost 0.8 per cent. The formation of some allyl thiocyanate (*34, p. 832*), allyl cyanid (*13*), and carbon bisulphid during the fermentation process of sinigrin has been observed in experiments where no alcohol was present. Other products may be formed in its presence and must be expected, especially in the authors' material, where no sinigrin but another glucoside is present. It has been pointed out by Kuntze (*25*) that side reactions can occur between allyl isothiocyanate and alcohol with the formation of allyl thiourethane, and it may be presumed that a similar reaction might take place between crotonyl isothiocyanate and alcohol, which will lead to erroneous but probably lower results. At the present time the data obtained are insufficient to ascribe the discrepancy to any of these causes, nor is it yet known whether, in the presence of alcohol, potassium hydrogen sulphate has also an injurious effect upon the formation of mustard oil through rendering myrosin largely ineffective by causing its coagulation (*12*). It is believed, however, that the maximum yield was obtained, since, even in the presence of large amounts of enzym (white mustard added), higher yields were not secured.

Shaking the maceration mixture at room temperature at intervals of five minutes did not hasten the reaction sufficiently to give the total amount of volatile oil in two hours.

It has been pointed out by Förster (*11*) that in the preparation of rapeseed cake the material is heated to about 70° C., and that when it is so treated a high yield of oil is obtained. In these experiments the authors were unable to verify Förster's results. In order to see whether there was any difference in the yield of oil from the brown and yellow seeds, a separation of the two was made, and determinations were made on the separated samples with the results (calculated as crotonyl isothiocyanate) shown in Table IV.

TABLE IV.—*Relative yield of volatile oil by brown and yellow seeds of Chinese colza.*

Treatment.	Yield of volatile oil.	
	Brown seed.	Yellow seed.
	Per cent.	*Per cent.*
2 hours' maceration at 37° C	0.58	0.55
½ hour's heating at 70° C., followed by 2 hours' maceration at 37° C	.44	.46

It will be observed that in both the brown and yellow seed a lower yield was obtained by the preliminary heating at 70° C. for ½ hour.

CHARACTER OF FIXED OIL, AND CERTAIN OF ITS CONSTITUENTS

It has been reported that the seed of Chinese colza is used in China for its fixed oil, and such use has also recently been made of the seed imported into this country. A study of the fixed oil showed that it was present in very large amounts, up to 50 per cent or more, and that its composition was similar to that of rape oils. It was a light yellow oil and apparently was of an excellent quality. The oil expressed from the seeds showed the characteristics given in Table V.

TABLE V.—*Characteristics of fixed Chinese colza oil* [1]

Density, 25° C	0.9097
Iodin No. (Hanus)	100.3
Saponification No.	173.8
Percentage of insoluble acids and unsaponifiable matter	96.1
Percentage of soluble acids	.07
Neutralization value of insoluble acids	172.6
Mean molecular weight of insoluble acids	325.0
Refractive index, 25° C	1.4695
Iodin No. insoluble acids	104.0
Percentage of solid acids	19.52
Percentage of liquid acids	75.82
Iodin No. solid acids	55.21

[1] Analysis by L. B. Burnett, formerly of the Oil, Fat, and Wax Laboratory, Bureau of Chemistry United States Department of Agriculture.

CHEMICAL CONCLUSIONS

1. The volatile oil yielded by Chinese colza has been identified as crotonyl isothiocyanate, an oil formerly found in rape. Crotonyl isothiocyanate is slightly lighter and allyl isothiocyanate slightly heavier than water. The boiling points of the oils and other physical constants referred to in Table II permit ready differentiation.

2. The yield of volatile oil varied from about 0.4 to 0.6 per cent, while true mustards, with the exception of *Sinapis alba*, yielded from about 0.7 per cent up to more than 1 per cent of volatile oil of different composition.

3. The fixed or fatty oil expressed from the seed showed the general characteristics of rape oils, these being slightly different from the fixed mustard oils. The iodin value, for instance, was about 100 or below in case of rape oils and the Chinese colza oil, while it was above 100 in the case of oil expressed from different mustard species. These oils were obtained under similar conditions and were unrefined. Methods of refining may change the iodin value.

4. The yield of fixed oil varied from about 40 to 50 per cent, whereas the true mustards examined usually contained less and rarely, if ever, more than 40 per cent of a fixed oil.

PHYSIOLOGICAL DATA

GENERAL PHYSIOLOGICAL CHARACTERISTICS

Chinese colza seed when chewed has an earthy and slightly pungent taste, the flavor being suggestive of cabbage or turnip rather than of mustard. When a few grams of the freshly triturated seed macerated with water have stood in a closed vessel at room temperature for a few minutes, the odor of the volatile oil formed may readily be noted. This odor gradually becomes weaker, however, and after the mixture has stood for 24 hours, more or less, the odor is largely gone and is often replaced by an odor of hydrogen sulphid. The mustards (except white mustard) give the characteristic mustard oil flavor and irritating sensation to the membranes of nose and eyes much more strongly and for a longer period. In fact, while the vapor of true mustard oil, even in very small amounts, causes great discomfort to eyes, nose, and lungs, the effect of the vapor of crotonyl isothiocyanate was by no means to be compared in intensity and hardly in character.

The ground seed, with small amounts of water added, was applied in the form of a plaster to the skin of the arm. No more than a reddening was caused after 2 or more hours of application; no blistering whatsoever was noted, and the reddening soon disappeared. It was necessary to remove plasters prepared similarly with true mustards after shorter application, and blisters were left. When applied to the skin the isolated volatile oil itself caused only a burning sensation and a temporary reddening.

PHARMACOLOGICAL ACTION

While no pharmacological experiments were made in this investigation, those of Sjollema and others may be briefly mentioned. Sjollema (*35, p. 315*) gave 0.212 gm. of the oil (crotonyl isothiocyanate isolated from rape) to a rabbit in the form of an emulsion but observed no abnormal symptoms. After about three hours the animal began to eat again, appeared entirely normal, and lived. Allyl mustard oil, isolated from black mustard, given in the same amounts and under comparable conditions, caused death to a rabbit within a few hours. The experiments were repeated with the same result. Stein (*37*) confirms Sjollema's findings in general upon the basis of a larger series of experiments, also with rabbits. He concludes that while the symptoms of poisoning are in a way the same as those caused by allyl isothiocyanate, the general toxic (resorptive) as well as the locally cauterizing properties are far less pronounced in the case of crotonyl isothiocyanate. The toxic dosis may be estimated to be 0.5 gm. of crotonyl, against less than 0.1 gm. of allyl isothiocyanate per kilo body weight.

He experimented also with a goat (24 kgm. in weight) adding to the feed of ground potato and sugar beets the following amounts of crotonyl

isothiocyanate: 1 cc. the first day, 1.5 cc. the second day, and 2 cc. the third day. Except for an increased urine excretion, no disturbance was observed; the urine was free from albumen. In experimenting with cattle, Moussu (*30*) used the pure oil of allyl isothiocyanate as well as cakes containing crotonyl isothiocyanate. Concerning allyl isothiocyanate given internally, he concludes that it is very toxic and may cause in doses of 2 gm. per 100 kilos speedy death, with the symptoms of an acute inflammation of the intestines.

No injury was observed from feeding large and varying amounts of rape-seed cakes, either dry or mixed with water, though cows of different ages were fed with the cakes which, according to Brioux (*6*), contained over ½ per cent of crotonyl isothiocyanate. One old cow, not in milking condition, weighing about 400 kgm., was fed in four periods of five days each the amount of 1 kgm. of cake per day, increasing to 3 kgm. per day. Apparently no injury was caused, although the cow received in the final period about 17 gm. of crotonyl isothiocyanate in the form of a cake, yielding 0.57 per cent of this oil. Brioux, on the basis of Moussu's experiments, concludes that allyl isothiocyanate is six or seven times as toxic as crotonyl isothiocyanate. He pointed however, to the fact that Moussu fed the volatile oil of allyl isothiocyanate directly, while previous authors, using the mustard cake, could feed without causing injury to the animal amounts which contained decidedly larger quantities of volatile mustard oil. For other experiments with allyl mustard oil see Carlier (*9*).

BACTERICIDAL ACTION

The bactericidal effect, so strong in the case of allyl mustard oil and so essential for the keeping qualities of prepared mustard, as Kossowicz (*24, p. 329*) and others have pointed out, is lacking or very weak so far as crotonyl isothiocyanate is concerned. Stein made the following interesting experiments.

To a number of test tubes containing 10 cc. of raw milk a small amount (on point of knife) of liver of sulphur and also increasing but definite amounts of allyl isothiocyanate and crotonyl isothiocyanate, respectively, were added. A test paper, moistened with lead salt solution, was fastened in the opening of the tube. The opening was then closed with cotton, and the tubes were set aside at from 38° to 40° C. for 24 hours. The blackening of the lead paper, caused by the bacterial formation of hydrogen sulphid and subsequent formation of lead sulphid, indicated in which experiments bacterial activity was not inhibited by the addition of either of the volatile oils. His results are given in Table VI.

The far greater bactericidal effect of allyl mustard oil is clearly evident. Further interesting data on the bactericidal action of allyl mustard oil and the amounts which prevent the growth of bacteria or yeasts belonging to different species are reported by Kossowicz (*23, p. 149–161*).

TABLE VI.—*Bactericidal action of allyl isothiocyanate and crotonyl isothiocyanate*

Allyl mustard oil.		Crotonyl mustard oil.	
Number of drops.[1]	Intensity of blackening.	Number of drops.[1]	Intensity of blackening.
1/20	10	1/20	10
1/10	10	1/10	10
1/5	8	1/5	10
1/3	1	1/3	10
1/2	0	1/2	10
1	0	1	10
.....	.....	2	10
.....	.....	3	5
.....	.....	4	5
.....	.....	6	3
.....	.....	8	2
.....	.....	10	0

[1] One drop = 0.05 cc.

Certain manufacturers of prepared mustard who unwittingly used the Chinese colza seed as mustard in the usual proportions in preparing their product noted extensive spoilage within a short time. The deficiency of crotonyl isothiocyanate with respect to its bactericidal action is thus also demonstrated in a practical and very impressive way.

PHYSIOLOGICAL CONCLUSIONS

Crotonyl isothiocyanate differs distinctly from true volatile mustard oil (allyl isothiocyanate). Its flavor resembles that of cabbage or turnip instead of that of onion. No appreciable effect on the eye or the mucous membrane of the nose and no blistering effect on the skin were noted, in contrast to such and other effects of allyl isothiocyanate. Crotony isothiocyanate lacks also the pronounced bactericidal qualities of true volatile mustard oil. Moreover, crotonyl isothiocyanate is distinctly different from the nonvolatile mustard oil, para-oxybenzyl isothiocyanate, of white mustard, which has no odor but has a strong biting taste and a strong blistering effect on the skin.

SUMMARY

Material imported as rape seed and sold as mustard seed was identified as Chinese colza, *Brassica campestris chinoleifera* Viehoever, n. var.

The characteristics of the seed have been established, and those which permit the identification and differentiation from true mustard seed have been pointed out.

Plants have been grown from the seed, and the characteristics have been established, especially with reference to their close relationship to the colza group, *Brassica campestris*.

The volatile oil obtained from the material was identified as crotonyl isothiocyanate, which is not a suitable substitute for mustard oil, in respect to condimental, bactericidal, or medicinal value.

The fixed oil proved to be of the general composition of the rape oils, and the quantity of the oil present, amounting to more than 40 per cent, characterized the seed as a very valuable oil seed.

On the basis of the general composition of the seed and the character of the volatile oil, it is suggested that the pressed oil cake may well be used as a stock feed.

The leaves are succulent and should be of value as greens.

The plants, which are very vigorous and apparently hardy, seem to offer possibilities as a forage crop. Experiments along this line have been undertaken in cooperation with the Bureau of Plant Industry.

CONDENSED DESCRIPTION OF CHINESE COLZA (BRASSICA CAMPESTRIS CHINOLEIFERA VIEHOEVER)

Basal or radical leaves first single, later numerous, arranged in cluster, large glossy green, usually smooth, obovate or round, obovate in general outline, entire or obscurely wavy, variously toothed, sometimes crenate, tapering into a distinct thin petiole, which is more or less margined, showing sometimes a few leaflike lobes.

Leaves of flowering stem more or less glaucous, clasping, obovate, oblong, or somewhat lanceolate acuminate; leaves of secondary stem not always clasping.

Flowers light yellow, of medium size (generally that of mustard flowers), pedicels averaging well over ½ inch.

Pods rather large and long, tapering into conical beak (0.4 to 0.8 inch long); pod and beak together from 2 to 3 inches long; from 8 to 12 seeds in pod.

Seeds yellow and brown, yellow greatly predominating; somewhat compressed, oval, usually distinct ridge on ventral side, usually smooth brown, slightly reticulated, varying in size (from 1.69 to 2.07 mm.), weighing from 1.4 to 6.4 mgm. (1,000 seeds weighed 2.865 gm. and 500 cc. weighed 352 gm.).

SEED COAT.—Epidermis about 5 microns thick; when it is moistened shows no swelling, no polarization of light, or cell structure. Parenchyma almost completely compressed. Sclerenchyma (palisade cells), from 15 to 31 microns high and from 8 to 15 wide, strongly thickened at base and side, without pigment, inner wall smooth. Pigment layer consists of one row of cells containing pigment only in brown seeds. Protein layer is formed usually by one row of large cells (from 15 to 21 microns high and from 15 to 42 microns wide).

SEED.—Composition averages as follows: Over 40 per cent fatty oil (colza or rape oil type); about 23 per cent protein (N = 6.25); 11.5 per cent reducing substances; 4 per cent crude fiber; 0.5 per cent by hydrolysis of volatile oil consisting of crotonyl isothiocyanate.

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PLATE 10

A.—Yellow seed of Chinese colza. Approximately × 5.

B.—Brown seed of Chinese colza. Approximately × 5.

C.—Surface section of yellow seed of Chinese colza, showing lack of reticulations. Approximately × 103.

D.—Surface section of brown seed of Chinese colza, showing reticulations. Approximately × 103.

E.—Cross section of yellow seed of Chinese colza. Approximately × 289.

F.—Cross section of brown seed of Chinese colza. Approximately × 289.

E and F show the following:

(a) Tangentially striated epidermis.

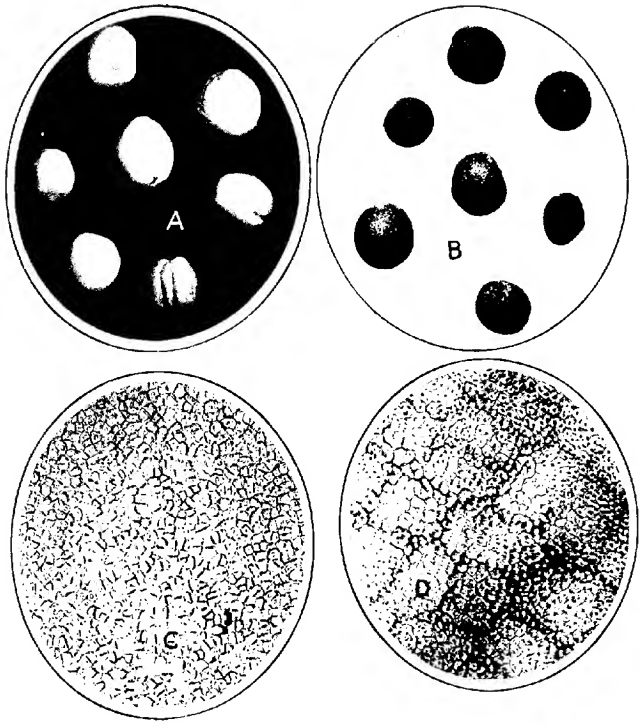
(b) Almost obliterated parenchyma.

(c) Sclerenchymatic palisade cells.

(d) Parenchyma, which in the brown seed (F) contains a pigment.

(e) Protein layer of the endosperm.

(f) Compressed parenchyma of the endosperm.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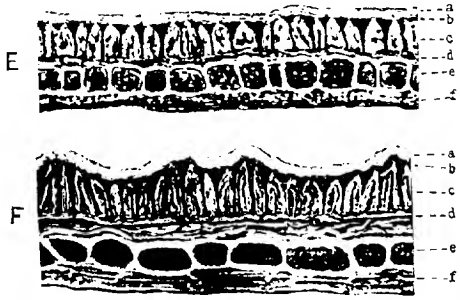
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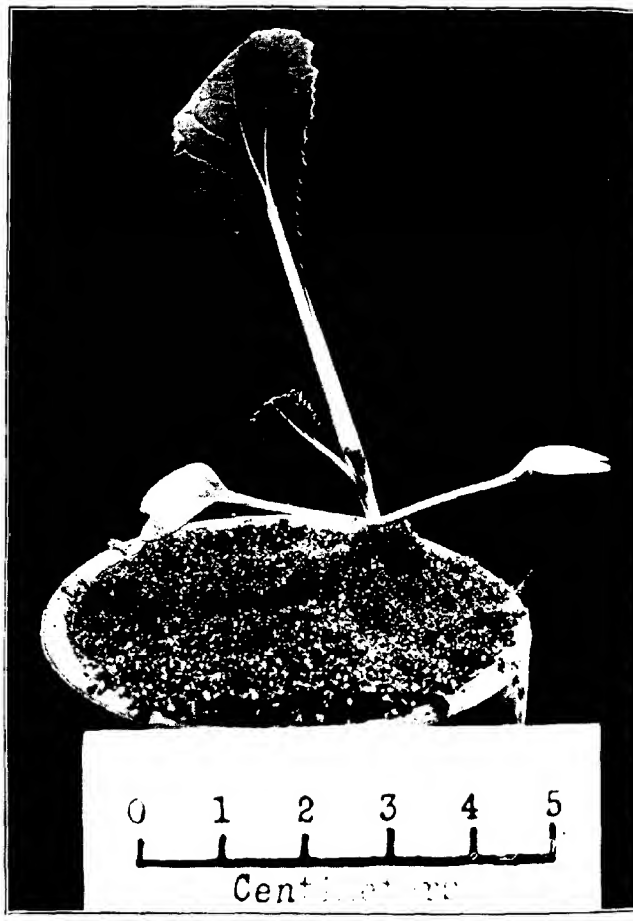
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PLATE 11

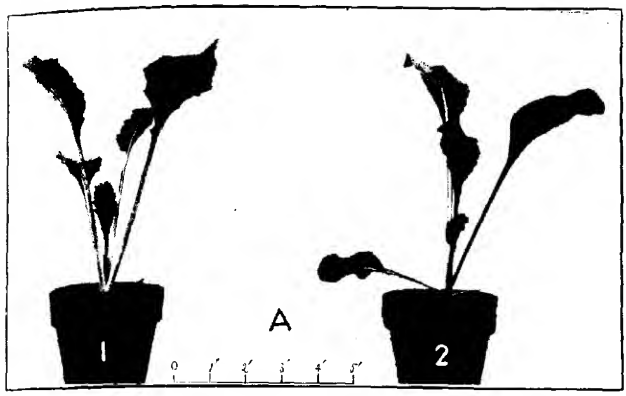
Seedling of Chinese colza, showing cotyledons and young leaves. The leaves show hairs, especially on the margin. Twenty-three days old. Grown in greenhouse. Planted March 23, 1917.

PLATE 12

Early rosette stage of Chinese colza seedling:

A.—Plants from (1) brown seed and (2) yellow seeds. Three weeks old. Grown in greenhouse. Planted March 14, 1917.

B.—Usual form, showing almost entire leaves. Three months old. Grown in greenhouse. Planted January 20, 1917.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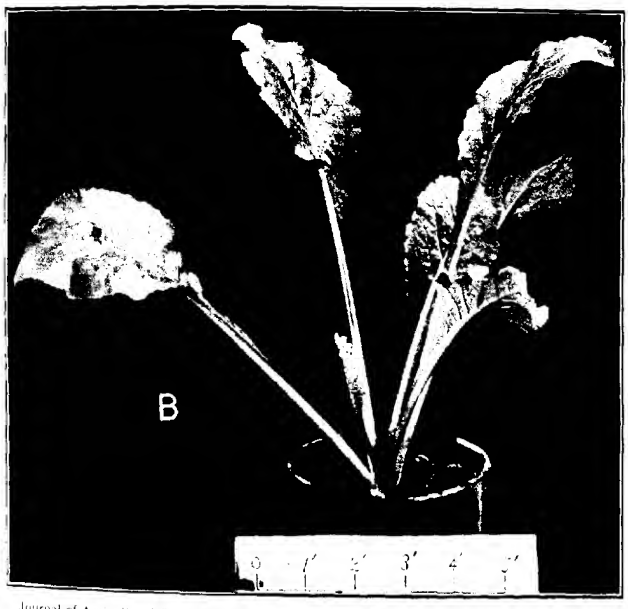
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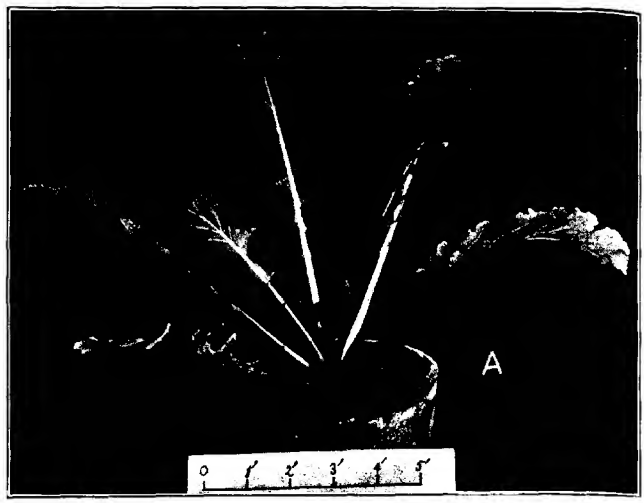
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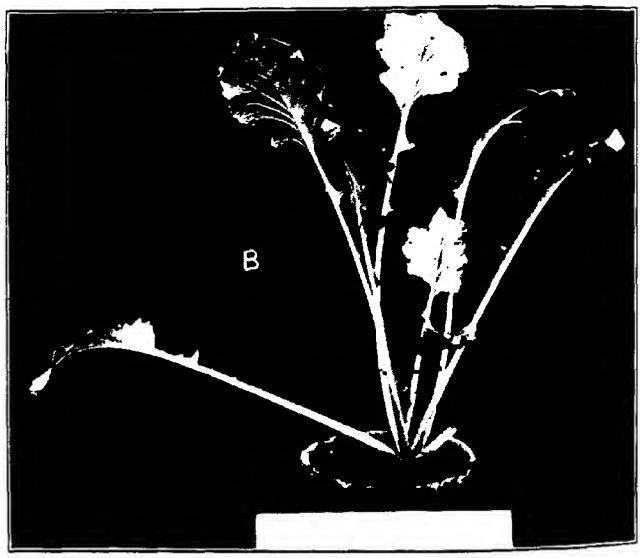

B

PLATE 13

Early rosette stage of Chinese colza seedling:

A.—Plant showing a variation in lobing of the leaves. Two months old. Grown in greenhouse. Planted February 20, 1917.

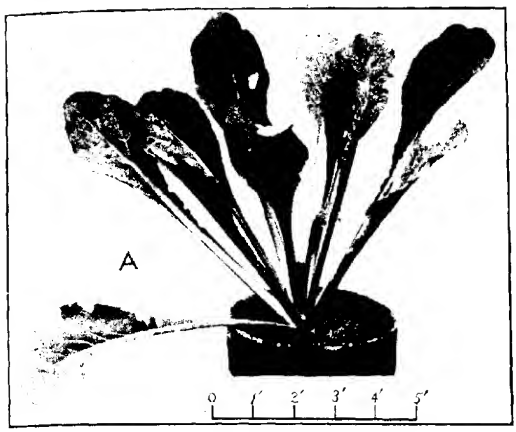
B.—Plant showing a variation in lobing of the leaves. Three months old. Grown in greenhouse. Planted February 20, 1917.

PLATE 14

Late rosette stage of Chinese colza seedling:

A.—Usual form. Three and one-half months old. Grown in greenhouse. Planted September 27, 1916.

B.—Plant showing a variation in lobing of the leaves. Two months old. Grown in field at Yarrow Station, Md. Planted May 16, 1917.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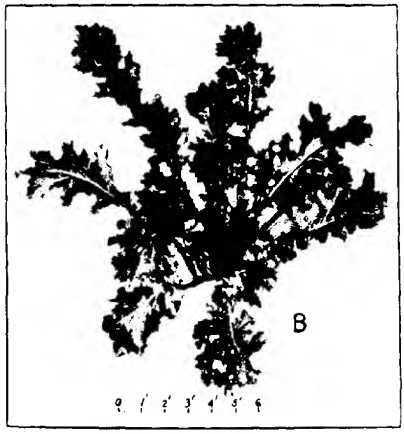
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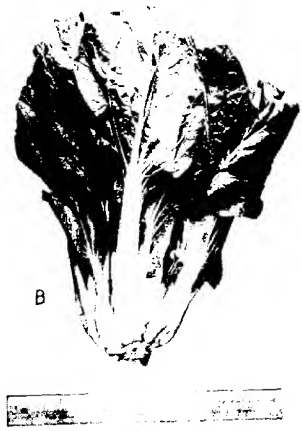
A


B


C

PLATE 15

Late rosette stage of Chinese colza seedling:

A.—Pe-tsai. Grown in field at Arlington, Va., by D. N. Shoemaker. The rule is 17¼ inches in length.

B.—Cross between Pak-choi and Pe-tsai. Grown in field at Arlington, Va., by D. N. Shoemaker. The rule is 15 inches in length.

C.—Pak-choi. Grown in field at Arlington, Va., by D. N. Shoemaker. The marked portion of the rule is 12 inches in length.

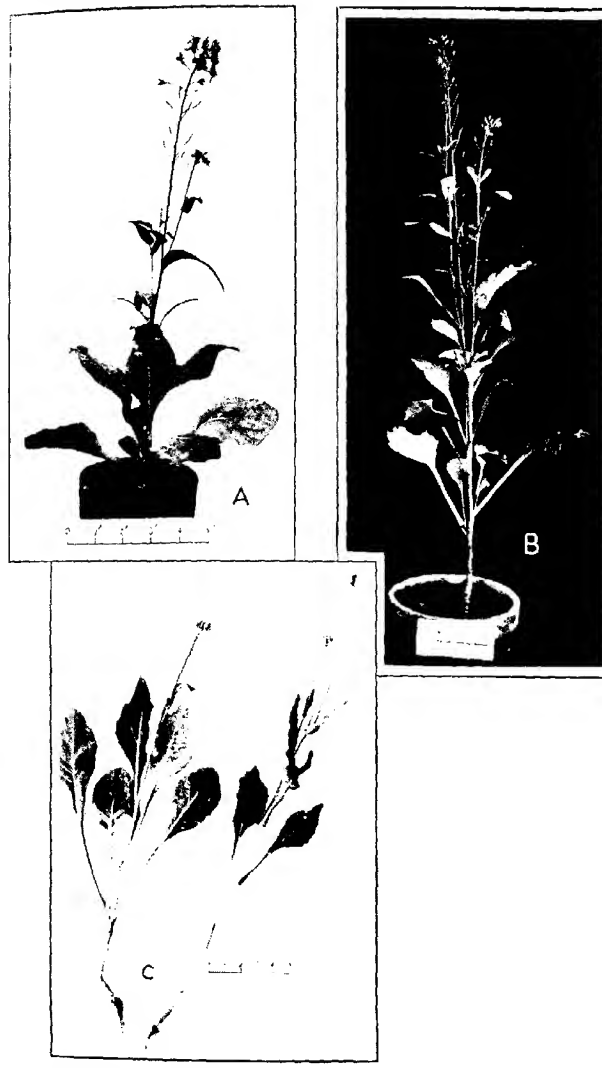
PLATE 16

Early flowering stage of Chinese colza:

A.—Usual form, showing somewhat enlarged stem base and stem-clasping leaves. Almost 5 months old. Grown in greenhouse. Planted September 27, 1916.

B.—Plant without enlarged stem base. Almost 5 months old. Grown in greenhouse. Planted February 20, 1917. The rule is 5 cm. in length.

C.—Usual form, showing glaucous leaves. Two months old. Grown in field at Arlington, Va. Planted about May 1, 1916.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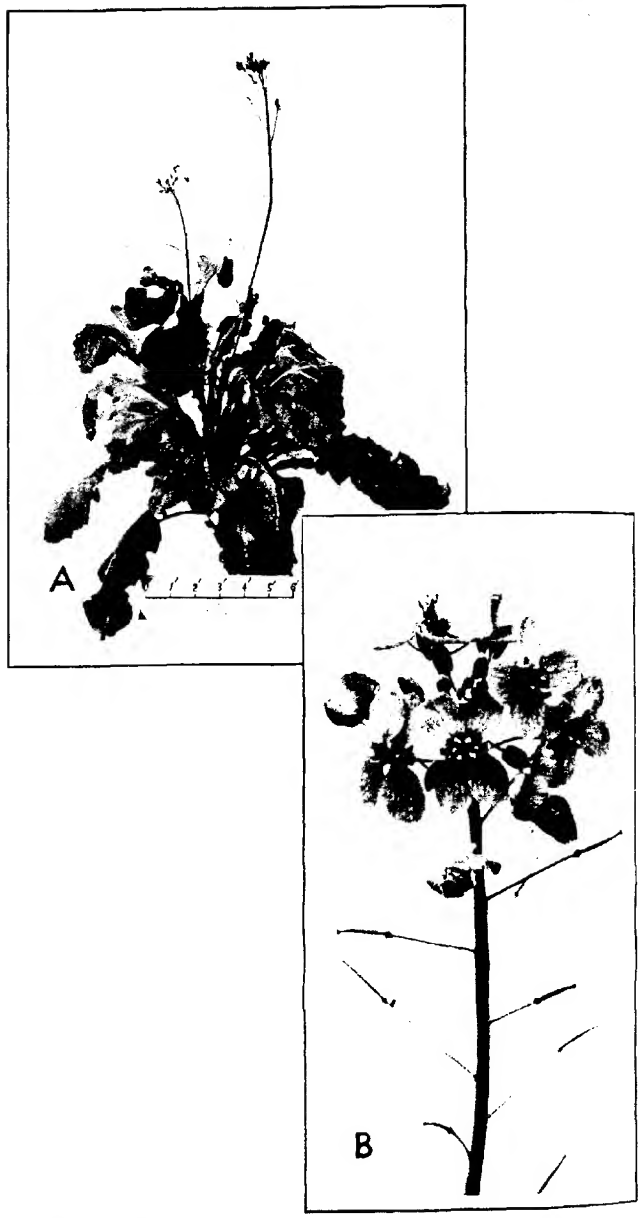
A
B

PLATE 17

Early flowering stage of Chinese colza:

A.—Usual form, showing luxuriant growth and long pedicels. Two and one-half months old. Grown in field at Yarrow Station, Md. Planted May 16, 1917.

B.—Flower cluster. Plant about 5 months old. Grown in greenhouse. Planted October 4, 1916. Natural size.

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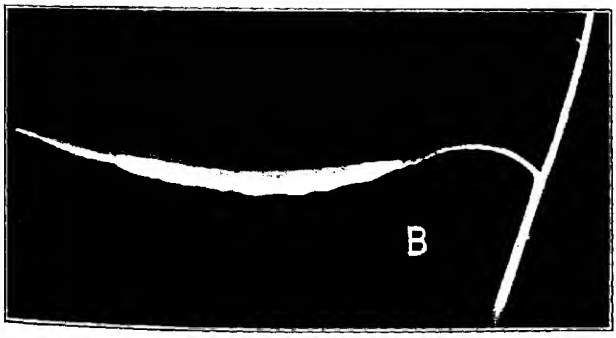
PLATE 18

A.—Fruiting stage of Chinese colza. Plant about 3 months old. Grown in field at Arlington, Va. Planted May 1, 1916.

B.—Mature fruit of Chinese colza. From a plant 7 months old. Grown in greenhouse. Planted October 4, 1916. Natural size.


A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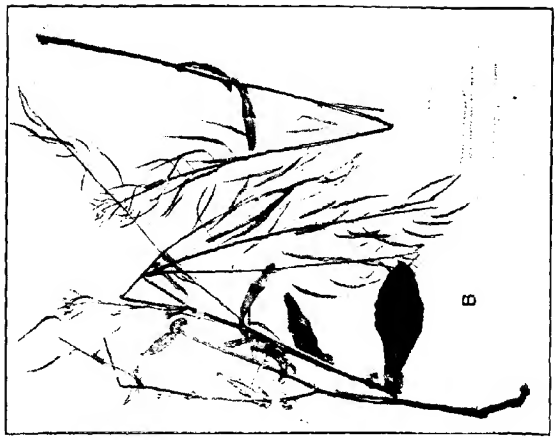
B


A

PLATE 19

A.—Herbarium specimen of *Brassica chinensis* L. Approximately $\times$ $^1/_5$.
B.—Herbarium specimen of *Brassica campestris*. Approximately $\times$ $^1/_3$.

STUDY OF SOME POULTRY FEED MIXTURES WITH REFERENCE TO THEIR POTENTIAL ACIDITY AND THEIR POTENTIAL ALKALINITY: I[1]

By R. F. KAUPP, *Investigator and Pathologist*, and J. E. IVEY, *Assistant in Poultry Husbandry, Research Laboratory of the Office of Poultry Investigations and Pathology, North Carolina Agricultural Experiment Station*

HISTORICAL REVIEW

Interest in the acid-base balance of dietaries has increased greatly in recent years. Sherman and his collaborators pointed out the basis for work of this kind when they made more accurate determinations than had hitherto been available of the ash constituents of the common feeds.

Sherman[2] has shown that meats and cereals have a preponderance of acid-forming elements, whereas, on the other hand, fruits and vegetables have an excess of base-forming elements.

It has been shown that ash has an influence on the reaction of the urine. Acid-forming feeds lead to the formation of more acid urines, and base-forming feeds cause the excretion of less acid or of alkaline urines. However, it has been found in studies carried on with men that certain exceptions were found—namely, plums, prunes, and cranberries, which, although yielding a basic ash, nevertheless increase the acid excretion because of the presence of benzoic acid, excreted as hippuric acid.[3]

Although the question whether or not an acid-forming diet eaten for some period of time is productive of undesirable results is debatable, probably the concensus of opinion is in favor of diets in which the acid-forming and base-forming elements are approximately balanced. The possibility that the continued use of acid-forming diets may lead to a greater susceptibility to disease of the less infectious type has seemed worthy of investigation.[4] Work along this line is in progress in this laboratory. Sour milk and buttermilk function as base because when used by the body the lactic acid is oxidized to carbonic acid, which is thrown off by the lungs, leaving a base residue of mineral salts. A common defect is the use of quantities of proteins and fats far in excess of the needs of the body. Proteins and fats are relatively expensive materials.

[1] This paper deals with the flocks for the first 24 weeks. Part II will deal with the first laying year.

[2] SHERMAN, Henry C. FOOD PRODUCTS. ix, 594 p., illus. New York, 1919.

[3] BLATHERWICK, N. R. THE SPECIFIC RÔLE OF FOODS IN RELATION TO THE COMPOSITION OF THE URINE. *In* Arch. Int. Med., v. 14, p. 409–450. 1914.

[4] HINDHEDE, M. PROTEIN AND NUTRITION . . . p. 8. London, 1913.

Journal of Agricultural Research,
Washington, D. C.
vf

Vol. XX, No. 2
Oct. 15, 1920
Key No. N. C.-14

THE PROBLEM

Profitable production of broilers begins with the baby chicks and extends over a period of about eight weeks, at the end of which time the birds should weigh, as a flock, approximately 1½ pounds each.

Our problem consisted of two parts:

1. To ascertain the mineral content of poultry feeds and, from this as a basis, to determine the potential acidity and potential alkalinity of these feeds.

2. To determine the acid-base balance of the feed mixtures used in our experimental feeding work in the production of broilers, giving some of the feeding results.

EXPERIMENTAL METHODS

The baby chicks were produced from a single flock of pure-bred Single-Comb White Leghorns, bred at the station and college poultry plant, and were hatched in the same incubator. Each lot was housed under similar oil-burning hovers of 100-chick capacity.

The experiment was carried on in periods of 8 weeks each and extended over three periods, or 24 weeks. The samples of feeds for analyses were obtained, as composite samples, from the various bags of feed used in the experiment. The potential acidity and potential alkalinity were estimated from the mineral analyses of the feed samples.

The caloric values of all the animal food rations—No. 2, 3, 4, and 7—are about the same, being 27 to 31 per cent protein calories. The soybean meal ration is in the same column so far as protein calories are concerned. The grain feeds have less protein caloric value, having only 12 per cent. Dried buttermilk functions as base. The meat scrap and bone meal and the digester tankage are base on account of the large amount of calcium in the bone. Blood is normally alkaline because of the sodium salts. The grain ration is 61.96 cc. acid per pound. The mashes are all alkaline or base. Those containing milk or bone—rations 2, 3, and 4—run high in base elements, while the blood meal runs only 41.21 cc. base and compares favorably with the two rations containing no animal food but containing protein from leguminous sources.

It will be noted that ration No. 6, the peanut-meal mixture, is low in protein caloric value. This is due to the fact that the peanut meal used was ground peanuts and hulls, not fat-extracted, and showed 40.4 per cent fat content.

Many interesting things are brought out by Table IV. The greatest gain in weight in chicks is during the first eight weeks, or the first period. Following the first period the increase in weight is gradually less during the remainder of the two periods.

The amount of feed required to produce a pound of gain gradually grows greater as the bird becomes older.

TABLE I.—*Mineral content of southern poultry feeds* [a]

[Results expressed in parts per hundred]

Kinds of feed.	Number of analyses.	Potassium.	Sodium.	Calcium.	Magnesium.	Sulphur.	Chlorin.	Phosphorus.	Iron.
Corn meal, bolted	4	0.349	0.072	0.0092	0.1336	0.160	0.0244	0.341	0.0040
Pinhead oats	7	.441	.109	.0126	.0794	.236	.0900	.499	.0019
Whole wheat	13	.435	.019	.0071	.1127	.183	.0630	.436	.0070
Wheat middlings	6	.949	1.219	.0980	.3628	.232	.0603	.785	.0052
Bone meal	3	.229	.735	21.1770	.5800	.170	.0900	10.349	.0180
Meat and bone meal	8	.158	.473	13.2927	.8333	.238	.5883	5.029	.1252
Rolled oats	2	.370	.136	.0430	.1500	.256	.0238	.473	.0062
Whole corn	13	.337	.041	.0127	.1051	.148	.0521	.293	.0044
Hulled oats	6	.387	.053	.0915	.1465	.204	.0870	.454	.1090
Velvet bean meal [b]	1	1.186	.141	.3600	.2080	.151	.2220	.764	.0126
Soybean meal	5	1.179	.422	.2901	.2503	.310	.0373	.481	.0241
Peanut meal	5	1.191	.422	.2401	.2106	.325	.0312	.567	.0271
Skim milk	2	.151	.144	.1530	.0019	.042	.0650	.136	.0036
Egg, including shell	3	.0103	.200	.6080	.0985	.395	.1500	.3021	.0103
Rape, green	3	.2510	.008	.0084	.0206	.035	.0930	.0102	.00000076
Limestone grit	2	.0000	.000	30.9700	6.6700	.000	.0000	.0000	3.3330
Oyster shell	2	.0000	.000	37.9510	.4200	.147	.0900	.0000	.37500
Dried buttermilk	4	.7084	.9531	1.3491	.8107	.084	.3650	.1691	.4301
Digester tankage	4	.3605	.1948	3.0147	1.1654	.124	.3483	1.2323	.10175
Dried blood	4	.0941	.3223	.3755	.2175	.281	.1381	.2864	.01650

[a] Average of all analyses to Mar. 1, 1920.

[b] Velvet bean meal and pod.

TABLE II.—*Potential acidity and potential alkalinity of southern poultry feeds*

Kinds of feed.	Base.[a]	Acid.[a]
Corn meal, bolted		21.75
Pinhead oats		121.94
Whole wheat		79.09
Wheat middlings	206.58	
Bone meal	2,104.60	
Meat and bone meal	565.13	
Rolled oats		108.21
Whole corn		44.76
Hulled oats		79.17
Velvet bean meal	62.17	
Soybean meal	156.01	
Peanut meal	94.90	
Skim milk	23.18	
Rape, green	15.33	
Limestone grit	11,943.80	
Oyster shell	8,782.10	
Dried buttermilk	803.96	
Digester tankage	789.89	
Dried blood	63.68	
Egg, including shell		2.55
Peas, dried	24.00	
Potatoes, sweet	24.00	
Potatoes, Irish	26.00	
Rice		43.00
Spinach	122.00	
Turnips	25.00	
Beans, dried	79.00	
Beets	50.00	
Bread, hard		38.00
Cabbage	22.00	
Carrots	38.00	
Fish, dried		36.00
Hominy		24.00
Lettuce	27.00	

[a] Expressed in excess cubic centimeters per pound of feed

The bird gradually, in these tests, consumed more grain as it grew older. Likewise it was found that as a pullet comes into laying it consumes a greater proportion of mash and again slackens in its mash consumption as it goes into a nonlaying period.

The consumption of more grain and less mash has a tendency to lessen the base balance or, if the balance is already acid, has a tendency to increase the acid balance.

It was noted that the cereals are decidedly acid while the by-products from the legumes are of a base reaction. Feeds containing by-products, such as soybean meal and peanut meal, have a tendency to add to the base balance.

It is noted that rations containing buttermilk, digester tankage, and meat scrap and bone meal give a base balance in all cases.

The soda in the blood makes the blood meal base but not so strongly so as digester tankage or meat and bone meal containing much bone.

Bone is rich in calcium and also contains other bases such as sodium. As stated before, sour milk functions as base.

TABLE III.—*Acid-base balance of rations 1 to 7*

Ration No.	Kinds of feed.	Amount.	Acid.[a]	Base.[a]	Percentage of protein calories.
		Pounds.			
1	Scratch feed:				
	Corn	100			
	Oats	100	61.96		12
2	Mash feed:				
	Wheat middlings	35			
	Corn meal	30			
	Ground oats	35			
	Dried buttermilk	35		231.4	27
3	Wheat middlings	35			
	Corn meal	30			
	Ground oats	35			
	Meat and bone meal	20		125.9	30
4	Wheat middlings	35			
	Corn meal	30			
	Ground oats	35			
	Digester tankage	18		153.7	30
5	Wheat middlings	35			
	Corn meal	30			
	Ground oats	35			
	Soybean meal	24		63.9	30
6	Wheat middlings	35			
	Corn meal	30			
	Ground oats	35			
	Peanut meal	41		54.5	19
7	Wheat middlings	35			
	Corn meal	30			
	Ground oats	35			
	Blood meal	14		41.21	31

[a] Expressed in excess cubic centimeters per pound of feed.

In this work no account has been taken of the amount of calcium entering the crop as grit and oyster shell. This will, in all probability, overcome the acid reaction, though without definite data this is a mere guess.

In a large table of studies of rations furnished by the medical staff of the United States Army, the percentage of protein calories ran from 10 to 18. In the present work the percentage of protein calories ran from 13 to 22, according to the estimate of the actual intake in each period. There is a possibility, however, that we will need to pay more attention to the source or kinds and quantities of protein calories, since we have shown in this work that those birds that received animal food, including milk, tankage, meat and bone meal, and dried blood were prepared by the storing up of the proper potential energy to begin heavy egg production very young, while those birds that did not receive animal food of any kind were not prepared and did not go into early heavy egg production.

TABLE IV.—*Relation of protein calories, amount of feed, and acid-base balance to pound of gain with rations 1 to 7*

Ration No.	Period.[a]	Amount of feed consumed.			Amount of feed per pound of gain.	Percentage of gain.	Excess acid.[b]	Excess base.[b]	Percentage of protein calories.	Test protein.
		Mash.	Grain.	Total.						
		Pounds.	*Pounds.*	*Pounds.*	*Pounds.*					
1 and 2	First	23.3	15.4	38.7	1.0	[c] 800		114.6	21	Buttermilk.
	Second	35.2	28.6	63.8	[d] 4.2	200		90.8	20	
	Third	30.3	54.5	84.8	[d] 6.0	63		42.8	17	
1 and 3	First	22.8	18.7	41.5	2.7	905		41.2	21	Meat and bone meal.
	Second	36.7	35.3	72.0	4.9	137		33.8	21	
	Third	34.8	61.9	96.7	4.9	50		5.6	17	
1 and 4	First	23.6	20.0	43.6	1.8	1,142		54.8	21	Digester tankage.
	Second	17.5	23.7	41.2	6.4	141		29.6	19	
	Third	14.3	22.2	36.5	6.6	62		22.5	19	
1 and 7	First	19.7	20.8	40.5	2.1	1,300	11.7		21	Blood meal.
	Second	33.8	33.1	66.9	5.8	83	9.8		21	
	Third	26.1	48.7	74.8	7.0	49	25.8		18	
1 and 5	First	19.5	14.5	34.0	2.9	900		10.2	22	Soybean meal.
	Second	31.3	36.9	68.2	5.9	133	4.1		20	
	Third	30.0	56.9	86.9	8.4	51	18.4		18	
1 and 6	First	32.7	21.9	54.6	3.1	800		7.8	16	Peanut meal.
	Second	30.9	27.3	58.2	4.7	206	1.1		15	
	Third	10.4	48.4	58.8	5.3	59	41.3		13	

[a] Each period consists of eight weeks.
[b] Expressed in excess cubic centimeters per pound of feed.
[c] Computed on the basis of weight of the baby chicks at hatching and at the beginning of each period.
[d] In the latter part of the third period, in all the flocks receiving animal food, egg production began. As egg production begins it is noted that the birds consume relatively more mash, which has a tendency to lessen the acid balance. If the birds are not in egg production, less mash and more grain are consumed, which increases the acid balance.

TABLE V.—*Acid-base balance of rations 8 to 11*

Ration No.	Kinds of feed.	Amount.	Acid.[a]	Base.[a]	Percentage of protein calories.
		Pounds.			
8	Rolled oats	8	.....	.....	.....
	Wheat middlings	8	.....	.....	.....
	Meat and bone meal	2	.....	.....	.....
	Bone meal	1	.....	128.3	29
9	Cracked wheat	3	.....	.....	.....
	Cracked corn	2	.....	.....	.....
	Pinhead oats	1	67.2	.....	13
10	Wheat middlings	6	.....	.....	.....
	Corn meal	3	.....	.....	.....
	Meat and bone meal	3	.....	.....	.....
	Bone meal	1	.....	382.6	30
11	Whole wheat	3	.....	.....	.....
	Cracked corn	2	.....	.....	.....
	Hulled oats	1	67.2	.....	13

[a] Expressed in excess cubic centimeters per pound of gain.

TABLE VI.—*Relation of protein calories, amount of feed, and acid-base balance, to pound of gain during first eight weeks*

Kinds of feed.	Amount.	Excess acid.[a]	Excess base.[a]
	Pounds.		
Milk	52.30	.....	1,212.31
Ration No. 8	2.42	.....	310.48
Ration No. 9	8.42	565.82	.....
Ration No. 10	4.30	.....	1,645.18
Ration No. 11	5.98	401.85	.....
Rape	7.81	.....	119.72

[a] Expressed in excess cubic centimeters in each feed consumed.

Amount of feed per pound of gain, 3.4 pounds.
Percentage of gain, 686.
Excess base intake, 2,320 cc.
Excess base intake per pound of feed, 85.5 cc.

SUMMARY

From Table II we note that corn, wheat, and oats, as well as egg with the shell, rice, bread, fish, and hominy are acid. The wheat middlings, in six analyses, on account of the high content of sodium and potassium, are base. Green feeds, such as rape, cabbage, carrots, beets, turnips, potatoes, spinach, and lettuce, are base. Seeds of the legumes, such as velvet bean, soybean, peanut, and peas, are base. Bone meal, on account of its high calcium content as well as other base-forming elements, is highly base. Limestone grit is very highly base, and also to a less extent is crushed oyster shell. The animal feeds containing bone, such as meat and bone meal and digester tankage, are base. The calcium of the egg shell does not quite overcome the acid of the albumin of the egg. Dried

milk functions as base because the lactic acid is oxidized to carbonic acid, which is thrown off by the lungs, leaving the basic residue of mineral salts. Dried skim milk or dried buttermilk is therefore quite base in function. Dried blood, on account of its magnesium, calcium, and sodium content, is moderately base.

In these studies there have been arranged 11 feed mixtures for acid-base studies. The first 7 are North Carolina Experiment Station formulae and the last 4 are those of Prof. Rice. The mixtures that contain considerable amounts of either dried milk, meat and bone meal, or digester tankage are quite base. The mixture containing soybean meal is approximately as much base as the grain mixture is acid, so that equal amounts would approximately balance so far as acid-base content is concerned. The peanut meal mixture is slightly below the soybean meal mixture, and the blood meal comes slightly below the peanut meal.

We note from Table III that the grain mixture contains 12 per cent protein calories and the ground feed mixtures contain from 19 per cent in the mixture containing peanut meal in which peanut meal not fat-extracted to 31 per cent in the ration in which blood meal was used. We note by a study of Table IV, which gives the total intake of each mixture for each period, that the final percentage of protein calories runs from 13 to 22. For comparison with rations for human beings we may again refer to the study of army rations during the late World War, in which the percentage of protein calories ran from 10 to 18. Dr. Osborne[1] found that 12.5 per cent protein calories produced maximum growth in rats. The indications are that the kinds as well as the quantities of proteins are essential factors. While the kinds of amino acids and vitamins are important factors in addition to kinds and quantities of minerals, there is a possibility that there are other factors undiscovered which have a profound bearing on growth, egg production, and the preparing of pullets, by aiding the storing up of potential energy, for early and heavy egg production. Data which will be published later show that pullets grown on range or in confinement without animal food of any kind, though the protein calories were above those indicated in comparison rations,[1] were not prepared for early heavy egg production and did not show high egg yields until animal food of some kind had been added. In this instance this was the soybean meal and peanut-meal lots. In the second and third periods the balances of intake was acid.

Further studies are being made to determine whether acid feeds will in any way interfere with either growth or egg production. In these studies rations 5 and 6 can be made base by the addition of ground limestone or gound oyster shell. The amounts to be added would depend upon the proportions in which the mash and grain were fed.

[1] OSBORNE, Thomas B., and MENDEL, Lafayette B. A QUANTITATIVE COMPARISON OF CASEIN, LACTALBUMIN, AND EDESTIN FOR GROWTH OR MAINTENANCE. *In* Jour. Biol. Chem., v. 26, no. 1, p. 9. 1916.

We find, by a study of Table V, that the grain rations 9 and 11 are acid and that the mash is base. In these mash mixtures there has been added both bone meal and meat and bone meal. Wheat middlings also aid in overcoming the acidity of corn meal and of rolled oats. In this test the total intake excess was base. The percentage of protein calories was 22.

CONCLUSIONS

Grain mixtures as ordinarily used in poultry feeding are acid.

Mash mixtures containing sufficient quantities of digester tankage, meat and bone meal, dried milk, or dried blood will be base.

Acid balances of feed mixtures can be overcome by the addition to mashes of dried milk, digester tankage, meat and bone meal, bone meal, dried blood, or ground limestone or oyster shell. Green feed, milk to drink, and limestone and oyster-shell grit also aid in overcoming the acid balance of grain mixtures.

THE INFLUENCE OF COLD IN STIMULATING THE GROWTH OF PLANTS [1]

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In regions having a cold winter like ours, with prolonged or repeated freezing, the native trees and shrubs become dormant in autumn. According to the general belief this condition is brought about by the cold. It is also the general belief that warm weather is of itself the sufficient cause of the beginning of new growth in spring. Both these ideas are erroneous. It is the object of the present address to show: first, that in our native trees and shrubs dormancy sets in before cold weather, and that cold weather is not necessary for the establishment of complete dormancy; second, that after such dormancy has begun, the exposure of the plants to an ordinary growing temperature does not suffice to start them into growth; third, that these plants will not resume normal growth in the warm weather of spring unless they have been subjected previously to a period of chilling; and, finally, a theory will be advanced to explain this paradoxical effect of cold in stimulating growth instead of retarding it.

The subject will be presented in a series of numbered statements, each followed by supporting evidence.

1. TREES AND SHRUBS OF COLD CLIMATES BECOME DORMANT AT THE END OF THE GROWING SEASON WITHOUT THE NECESSITY OF EXPOSURE TO COLD WEATHER.

A little more than 10 years ago, while engaged in a series of greenhouse experiments, the speaker came upon a strange phenomenon which was wholly unexpected and which threatened to interfere seriously with the success of the experiments. Healthy blueberry plants, intended to be used during the winter for breeding purposes, were brought into the greenhouse at the end of summer and were kept at an ordinary growing temperature. They refused to continue their growth during the autumn, gradually dropped their leaves, and went into a condition of complete dormancy. They did this at a greenhouse temperature which in spring and summer would have kept the plants in a condition of luxuriant growth. The completeness of the condition of dormancy which such plants reach can be best appreciated from photographs (Pl. 20, A).

Since 1910 this experiment has been repeated many times and with many species of plants, and without exception those trees and shrubs

[1] An address delivered before the National Academy of Sciences Apr. 27, 1920.

Journal of Agricultural Research, Washington, D. C.
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Vol. XX, No. 2
Oct. 15, 1920
Key No. G-205

native of our northern cold-winter region which were tested went dormant in fall or winter regardless of temperature. In comparing outdoor plants with indoor plants of the same species the most that can be said in favor of outdoor conditions is that dormancy progresses a little faster in outdoor plants, evidently because their foliage is injured by freezing weather, and they drop their leaves somewhat earlier than indoor plants.

2. TREES AND SHRUBS THAT ARE KEPT CONTINUOUSLY WARM DURING THE WINTER START INTO GROWTH MUCH LATER IN SPRING THAN THOSE THAT HAVE BEEN SUBJECTED TO A PERIOD OF CHILLING.

In the late winter and early spring of 1910 I waited patiently, and then impatiently, for my indoor plants to bloom, and at last I was forced to realize that they never would bloom. When compared with plants of the same kind that had been outdoors during the winter and had been brought into the greenhouse in early spring, the difference was astonishing. The outdoor plants burst into leaf and flower luxuriantly, while the indoor plants remained completely dormant and naked. The experiment was repeated many times and with various species of plants, some of which may be used in illustration. (See Pl. 20, B; 21; 22, A.)

At first it was supposed that the plants needed to be frozen to start them into growth, but a single freezing proved not to be effective. And then it was found that the dormant plants would start into growth without any freezing whatever. It was necessary only that they be subjected to a period of prolonged chilling, usually two or three months, at a temperature a few degrees above freezing.

If plants are kept continuously in a warm place without chilling, the dormant condition often continues for an extraordinary length of time. In some instances plants have remained dormant for a whole year under conditions of heat, light, and moisture that ordinarily would make the same plant grow with the greatest luxuriance.

3. THE STIMULATING EFFECT OF COLD IS LIMITED TO SUCH PORTIONS OF THE PLANT AS ARE SUBJECTED TO THE CHILLING.

The conspicuous difference in spring growth between chilled plants and plants not chilled has already been shown. These differences, furthermore, can be produced experimentally upon different parts of the same plant. Plants thus treated present a very curious and remarkable appearance, as shown in Plate 22, B, and Plate 23.

On February 3, 1912, a blueberry plant (Pl. 22, B) 44 inches in height, which had shed its leaves and become dormant in a warm greenhouse maintained at a temperature of 60° to 70° F., was subjected to the following experiment: It was repotted in a 7-inch pot and set in the south end of a greenhouse at the temperature already mentioned. A small opening was made in the glass, and through this opening one of the two stems of the plant was pushed. The open space about the stem where it passed through the glass was carefully plugged with moss. During

the rest of the winter the plant remained in the same position, the pot and the stem, shown at the left in the illustration, continuing in the warm temperature of the greenhouse, while the stem at the right, projecting through the glass, was exposed to the rigors of winter, with its alternate freezing and thawing. The illustration, from a photograph made April 18, shows that when spring came the outdoor branch started into normal growth while the indoor branch continued dormant.

A second illustration (Pl. 23) shows a modification of the first experiment. In this case the plant was set on a shelf outside the greenhouse, and a single branch was passed through the glass wall into the warm interior. When spring came it was this interior branch that remained dormant, all the outside branches putting out leaves promptly and normally.

From a comparison of the two experiments it is evident that the difference in behavior of the indoor and outdoor branches could not have been caused by any special action of the root system, for in one experiment the roots were inside, in the other outside. It is clear that the causes that stimulated growth in the exposed stems operated in the stem itself, not in the roots. This principle is still further exemplified and confirmed by the behavior of cuttings taken from blueberry plants in the first stages of their dormancy. Such cuttings if kept warm continue their dormancy into late spring or summer, but if chilled for two or three months they start into growth at the normal time in early spring.

It should be stated here that the difference in the amount of light inside and outside the greenhouse had nothing to do with the stimulation to growth, for chilled plants are ready to start into growth promptly whether the chilling is done in the full light of an outdoor situation, or in the partial light of a greenhouse, or in the complete darkness of an ordinary refrigerator.

4. THE STIMULATING EFFECT PRODUCED ON DORMANT PLANTS BY COLD IS INTIMATELY ASSOCIATED WITH THE TRANSFORMATION OF STORED STARCH INTO SUGAR.

In most of our wild species of trees and shrubs the reserve carbohydrate material is stored away during summer and autumn in the form of starch. At the beginning of dormancy the twigs and sapwood are gorged with this material, the starch grains being stored ordinarily in the cells of the medullary rays and sometimes in the pith. As the process of chilling goes on, this starch little by little is transformed into sugar. The presence of large quantities of starch in the fall and early winter may be observed by applying to freshly cut surfaces of the twigs the well-known starch test of a 2 per cent solution of iodin in a 1 per cent solution of iodid of potassium. With a strong hand lens the starch is readily observed, if present, by the deep blue color it assumes under this treatment. The intensity of the coloration gives roughly an idea of the

number of starch grains present, and thus by this simple means anyone may observe in the twigs of trees and shrubs the gradual disappearance of their starch as spring approaches.

The measurement of the increasing amount of sugar is more difficult and must be done by chemical analysis. Through the courtesy of the Chief of the Bureau of Chemistry, exact data can be presented on this point from analyses by Mr. Lorin H. Bailey. In samples of dormant blueberry wood taken in early spring when growth was about to begin the ratio of sugar to starch proved to be seven times what it was in similar dormant wood taken in autumn.

I desire at this time to comment on the fact that one of my colleagues reading the manuscript outline of this address criticized the use of the word "stimulate" as applied to the effect which chilling produces on these dormant plants. His idea was that the chilling induced certain physiological changes in the cell contents but that the actual stimulation to growth came from the temperatures that followed the chilling. I defend, however, the propriety of the language I have used, for although the later stages of growth admittedly can not take place without warm temperatures, not only does the transformation from starch to sugar take place at the chilling temperature but the buds actually swell and push if the chilling temperature is continued for several months. In illustration I may cite the following experiments.

On March 3, 1915, 286 cuttings were made from dormant outdoor blueberry plants. They were stored in bundles, some in moist sphagnum moss, others in moist birch sawdust, at a contemplated temperature of 31° F., just below freezing. The cuttings remained in cold storage until December 6, a little more than nine months. An examination of the cuttings on that date showed that one or more buds had begun to swell on every cutting with the exception of a small number which were mildewed and dead. In other words, growth had already begun to take place at the cold-storage temperature. The thermograph record for the 278 days was as follows:

	Hours.
29° to 32° F.	5,591
32° to 33° F.	990
33° to 34° F.	91

The temperature record did not go above 34° F. It is an astonishing fact that temperatures so very near freezing will start dormant plants into growth.

On March 3, 1915, 58 cuttings from dormant, outdoor blueberry plants were placed in moist birch sawdust in commercial cold storage at 33° to 36° F. On December 4, nine months later, buds on every cutting had begun to grow. Not one of these cuttings gave a starch reaction when tested with iodin. The transformation of their stored starch into sugar was complete. (See Pl. 24.)

5. THE THEORY ADVANCED IN EXPLANATION OF THE FORMATION OF SUGAR DURING THE PROCESS OF CHILLING IS THAT THE STARCH GRAINS STORED IN THE CELLS OF THE PLANT ARE AT FIRST SEPARATED BY THE LIVING AND ACTIVE CELL MEMBRANES FROM THE ENZYM THAT WOULD TRANSFORM THE STARCH INTO SUGAR, BUT WHEN THE PLANT IS CHILLED THE VITAL ACTIVITY OF THE CELL MEMBRANE IS WEAKENED SO THAT THE ENZYM "LEAKS" THROUGH IT, COMES IN CONTACT WITH THE STARCH, AND TURNS IT INTO SUGAR.

I have stated the theory in these words out of regard for simplicity and general understanding, but if anyone should require that it be presented in orthodox technical language it might be restated as follows: The reserve amylum carbohydrate bodies are isolated from the amylolytic enzym by semipermeable protoplasmic living membranes of high osmotic efficiency, but under the influence of low temperatures the protoplasmic membranes are proximately devitalized, they become permeable to the amylolytic enzym, and amylolysis ensues. I may add, however, that the use of such terminology seems to me to involve a certain degree of unnecessary cruelty.

From the evidence already presented, no one, presumably, will question that the chilling of dormant trees and shrubs is followed by growth and that the growth is associated with the transformation of starch into sugar. But the hypothesis that this transformation is brought about by the weakening of the cell membrane and the consequent leakage of starch-transforming enzyms into the starch chambers may very properly be challenged. In the Tropics there is no chilling weather, yet trees and shrubs spring into growth after the dormant period of the dry season just as they do in temperate climates after the dormant period of winter. The critical scientific man will therefore ask, "Are there not other agencies than chilling which will start dormant trees and shrubs into growth even in our latitude?" It must be said in reply that there are. And it will be worth while to consider some of these causes, for not only are they of interest in themselves but also, instead of weakening the hypothesis here presented, they serve to strengthen and confirm it.

The data may best be presented through a series of illustrations.

The pruning of a long-dormant plant will often start it into growth (Pl. 25, A). Girdling produces a similar result (Pl. 25, B, at left). Notching the stem does the same (Pl. 25, B, at right). Rubbing the stem also starts the plant into growth (Pl. 26).

In all these examples of the stimulation of growth by injury it is conceived that the enzym is brought into contact with the starch as a direct result of the breaking and straining of the cells. Sugar is then formed and growth begins.

It should be observed that when a normal chilled plant starts growing it grows from many buds (Pl. 27, A), for the effect of the chilling on sugar formation is general. When a dormant plant starts growing as the result

of injury, however, it usually starts, as shown in several illustrations already presented, from a single bud, the one nearest the point of injury. The injury is local, and both the sugar formation and the growth that follows it are local.

We are now brought to the consideration of a phenomenon which I take to be of special significance—namely, the procedure by which the dormant plant starts itself into growth in the absence of chilling. After a blueberry plant has remained dormant at a warm temperature for a very long period, sometimes a whole year, the tips of the naked branches begin to lose their vitality. Just before or just after the death of the tip a single bud, or sometimes two buds, situated next below the dead or dying part starts growing (see Pl. 27, B; 31, A). The new growth of the stem is confined to the one or two buds, just as it was in the case of growth induced by injury. My interpretation of the phenomenon is that, as death approaches, the cell membranes become weakened in much the same way as when chilled, the enzym passes through into the starch storage cells, sugar is formed, and the adjacent bud begins to grow. The process going forward here in a restricted portion of the stem, and due to a local cause, is essentially the same as that taking place generally over the plant, from a general cause, when the plant is chilled.

In the Tropics some plants are able to grow continuously; others become dormant in the dry season and start into growth again at the coming of the rainy season. Tropical plants probably have various methods of coming out of their dormancy, and there is every reason to expect that some of them will be found to accomplish this act in the same way as our long dormant greenhouse plants, by the weakening of their cell membranes. This, I have endeavored to show, is in its effect substantially identical with chilling.

6. THE TWIGS OF TREES AND SHRUBS AFTER THEIR WINTER CHILLING AND THE TRANSFORMATION OF THEIR STARCH INTO SUGAR MAY BE REGARDED AS MECHANISMS FOR THE DEVELOPMENT OF HIGH OSMOTIC PRESSURES WHICH START THE PLANT INTO GROWTH.

Food in the form of starch can not be utilized by a plant directly. The starch must be changed into sugar before it can be used in making new growth. But this transformation does more than make the starch available as food for the growing plant. It serves also to increase the tendency of the cells to swell and enlarge. In the form of starch the material is inert in the creation of osmotic pressures, but when transformed into sugar it becomes exceedingly active. According to the rigid experimental tests of H. N Morse and his associates, a normal solution of cane sugar at 32° F. has an osmotic power of 25 atmospheres of pressure. It has been demonstrated that there sometimes occur in the cells of plants osmotic pressures as high as 30 atmospheres, or 450 pounds to the square inch, a pressure sufficient to blow the cylinder head off an

ordinary steam engine. It can hardly be questioned that these or even much lower osmotic pressures take an important part in forcing open the buds of once dormant plants.

We have evidence that there sometimes arise within the plant osmotic pressures of such intensity as to threaten the rupture of the cells. Consider the case of the exudation of drops of sugar solution from certain specialized glands. When this exudate of sugar occurs in flowers it is known as nectar, and it serves a useful purpose to the plant by attracting sugar-loving insects which unconsciously carry pollen from flower to flower and accomplish the beneficial act of cross-pollination. But sugar solution is often exuded outside the flower, in positions, or at times, that preclude any relation to cross-pollination. For example, a blueberry plant during its spring growth, when a leaf has reached nearly full size, is sometimes observed to exude drops of sugar solution from certain glands on the margins of the leaf and on the back of the midrib (Pl. 28). It is physically impossible for the sugar to have left the cells by osmosis. The sugar serves no useful purpose to the plant through the attraction of insects. The exudate certainly can not represent the elimination of a waste product, for sugar is one of the substances most used by plants in forming new tissues. I can conceive of no reason why the plant should exude sugar except to relieve a dangerous physiological condition—namely, the development of excessive osmotic pressures which would burst the cells of the plant or in some other way derange its physiological activities. I look upon such sugar glands as safety valves for the relief of excessive osmotic pressures that are dangerous to the internal economy of the plant. And not only is this conception applicable to extra-floral nectaries in general, but it may serve also, in the case of floral nectaries, to explain their origin. Having once arisen as osmotic safety valves, the usefulness of the floral nectaries as an aid to cross-pollination would then tend strongly to bring about their natural selection and perpetuation.

7. THE ESTABLISHMENT OF A DORMANT CONDITION BEFORE THE ADVENT OF FREEZING WEATHER AND THE CONTINUATION OF THIS DORMANCY THROUGH WARM PERIODS IN LATE FALL AND EARLY WINTER ARE PROTECTIVE ADAPTATIONS OF VITAL NECESSITY TO THE NATIVE TREES AND SHRUBS.

A little consideration will show how important the principle of chilling is to those species of trees and shrubs which are subjected each year to several months of freezing weather. If they were so constituted as to start into growth as easily in the warm days of late fall as they do in the warm days of early spring many species would come into flower and leaf in those warm autumn spells that we call Indian summer, and the stored food that the plant required for its normal vigorous growth in the following spring would be wasted in a burst of new autumn growth, which would be killed by the first heavy freezes and would be followed by a winter of weakness and probable death. But when two or three

months of chilling are necessary before a newly dormant plant will respond to the usual effect of warmth, such plants are protected against the dangers of growth in Indian summer. It is probable that all our native trees and shrubs are thus protected.

Any member of this audience may make a simple and instructive experiment next fall and winter with such early spring blooming plants as alder, hazelnut, pussy willow, yellow bush jasmine, forsythia, Japanese quince, peach, and plum. In mid-autumn bring into your living room and set in water freshly cut, dormant, leafless branches of these plants. They will not bloom. At intervals of a few weeks during late autumn and winter try the same experiment again. You will find that the branches cut at later dates will come into bloom under this treatment. They will not do so, however, until the expiration of the period of chilling appropriate to the various kinds of plants included in the experiment. The required period of chilling varies greatly. For some of the cultivated shrubs about Washington, especially the yellow bush jasmine (*Jasminum nudiflorum*), so brief a period of chilling is required that an extraordinarily cold period in late October or early November may chill them sufficiently to induce them to bloom if a period of warm weather follows in late November. The period of chilling required for the peach is so short that in Georgia unusually warm weather in December sometimes brings the trees into flower, and their crop of fruit is destroyed by the freezes that follow.

From these facts it appears that our native trees and shrubs are so intimately adjusted to the changes of the climate to which they have been long subjected that they are almost completely protected from injury by freezing, but some of the cultivated species brought from parts of the world having a climate different from ours are only imperfectly adapted to our climatic changes. They grow at times when our native species have learned to hold themselves dormant, and they often suffer severely in consequence.

Chilling, as a protective adaptation, has become a physiological necessity in the life history of cold-winter trees and shrubs. So fixed indeed, is the habit that it appears to be a critical factor in determining how far such plants may go in the extension of their geographic distribution toward the Tropics. In the Tropics our common northern fruit trees, apples, pears, peaches, cherries, grow well for a time and then become half dormant. In the absence of chilling they never fully recover from their dormancy; they grow with weakened vitality and finally die. If these fruits are to be grown successfully in the Tropics they must be given artificially the periodic chilling they require.

When it became evident from the earlier observations and experiments that chilling played so essential a part in the behavior of our trees and shrubs, it was clear that additional experiments ought to be conducted in which actively growing plants might be subjected to chilling

temperatures without being put in a dark place like the ordinary refrigerator. To meet the requirement of both cold and light a glass-covered, outdoor, brick chamber was constructed in 1912. It was kept above freezing by heating with electric lights, which were turned on and off automatically by a simple thermostat. In summer the chamber was kept cool, though not really cold, by means of ice and electric fans. Although much was learned with this apparatus, it was crude and inadequate. To provide for more exact experiments a glass-covered compartment chilled by a refrigerating machine was constructed in one of the Department of Agriculture greenhouses. The refrigerating apparatus is a sulphur-dioxid machine having a refrigerating power equivalent to 1,000 pounds of ice a day. It is run by a 2-horsepower electric motor, and it furnishes ample refrigeration for the lighted compartment, which is a glass-covered frame 25 feet long, 3 feet wide, and 14 to 20 inches in depth. The first of these refrigerated frames was devised and constructed in 1916. In this enterprise I had the valued advice and assistance of Dr. Lyman J. Briggs. The usefulness of this refrigerated frame in experimental work with plants was so great that another similar equipment was installed in 1918.

With the aid of this apparatus many of the experiments described in this address have been carried on or verified, as well as other experiments of a related character. For example, at ordinary summer temperatures many kinds of seed will not germinate but remain dormant until death overtakes them. Under the influence of chilling, however, these seeds are stimulated to prompt germination. (See Pl. 29.)

The experiments thus far made indicate the importance of a much wider use of the principle of chilling in many lines of experimentation bearing on the improvement of horticultural and agricultural practices. I commend the subject of chilling to experimenters in these lines, and I wish to call especial attention to the desirability of determining proper temperatures for the storage of seeds, bulbs, cuttings, and grafting wood, proper temperatures for the treatment of plants which are to be forced from dormancy to growth at unusual seasons, and proper temperature for the storage of nursery stock so that the nurseryman may have plants in proper condition for shipment on any date he desires. (See Pl. 30; 31, B; 32.)

The whole question of the effect of chilling on herbaceous perennials is an open field.

An understanding of the process of chilling explains the reason of some of the practices of gardeners, which they as well as botanists have erroneously ascribed to the need of "resting." What a gardener calls "resting" is often in reality a period of chilling, characterized not by physiological rest but by pronounced internal activity. Rest alone would not, in the case of our cold-climate trees and shrubs, accomplish the purpose the gardener has in mind. It is chilling, not rest merely, that

is required. The practice of gardeners and nurserymen known as the "stratification" of seeds is probably to be explained as in reality a process of chilling.

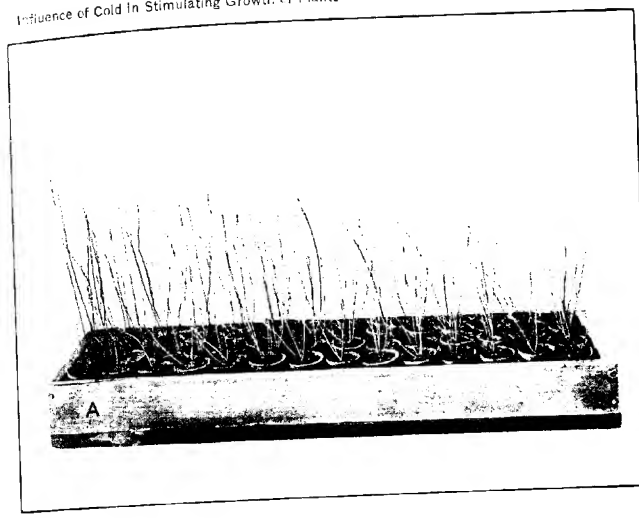
As a single example of the application of the principle of chilling let me cite the case of the blueberry. For several years we have been trying at the Department of Agriculture to domesticate this wild plant. We have raised many thousand hybrids and have set them out in waste sandy lands in the pine barrens of New Jersey (Pl. 33, A). We have grown the bushes to fruiting age and have brought them into highly productive bearing (Pl. 33, B). We have made them fruit so lusciously and so abundantly that they have brought returns to the grower at the rate of more than $1,000 an acre. In a word, we have changed the blueberry from a small wild fruit the size of a pea to a fruit the size of a Concord grape, and we have made its culture a profitable industry. (See Pl. 34, 35.) These things we should not have been able to do unless we had first worked out the principle of chilling, an understanding of which was essential to our work of breeding and propagation.

In conclusion, I wish to express the opinion that the chilling of dormant trees and shrubs of temperate climates as a prerequisite to their resumption of normal growth in the spring ought to be recognized in books on plant physiology as one of the normal processes in plant life. These works should contain chapters on chilling, just as they now contain chapters on other fundamental factors and principles relating to the life history of plants. And especially in books on plant physiology in relation to agriculture should the subject of chilling be dealt with in detail, for when in the pursuit of agriculture we take plants from one part of the world to another, or undertake to grow them out of season, or attempt to propagate them in quantity by grafting or by other processes unknown in nature, we are greatly handicapped and limited in our operations if we do not understand the principles of a process so widely existent in nature and so indispensable to a large proportion of the plants of temperate agriculture as the process of chilling.

PLATE 20

A.—Blueberry plants, *Vaccinium corymbosum*, made dormant without cold. These blueberry seedlings, in 2-inch pots, were kept during the fall and winter in a greenhouse at a temperature of 55° to 70° F. Although this is a very favorable temperature for the growth of the blueberry, these plants shed their leaves and became completely dormant, just as they ordinarily do when exposed to the frost and cold of an outdoor fall and winter. The photograph was taken on January 25.

B.—Chilled and unchilled blueberry plants. The six blueberry plants at the left, after an outdoor winter chilling, were brought indoors on March 25, into a greenhouse having a temperature of 55° to 70° F., and were repotted. On April 20, when the photograph was taken, they had developed both leaves and flowers, while the six plants at the right, which had been in the same greenhouse at the same temperature all the fall and winter and were repotted on the same date as the others, were still completely dormant.


A


B


A

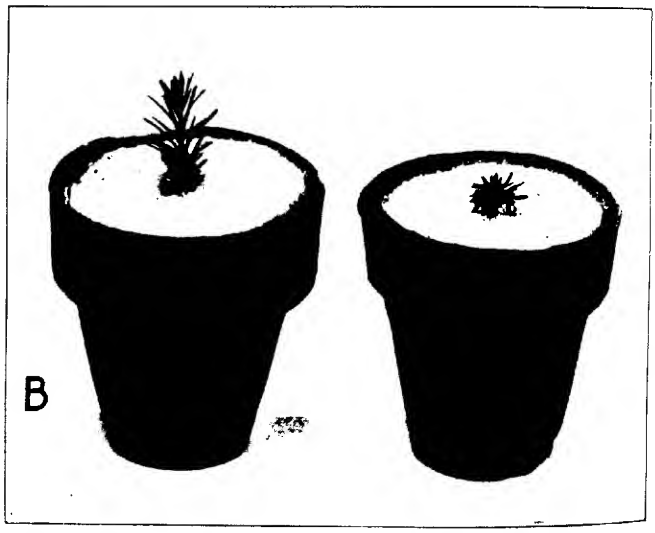

B

PLATE 21

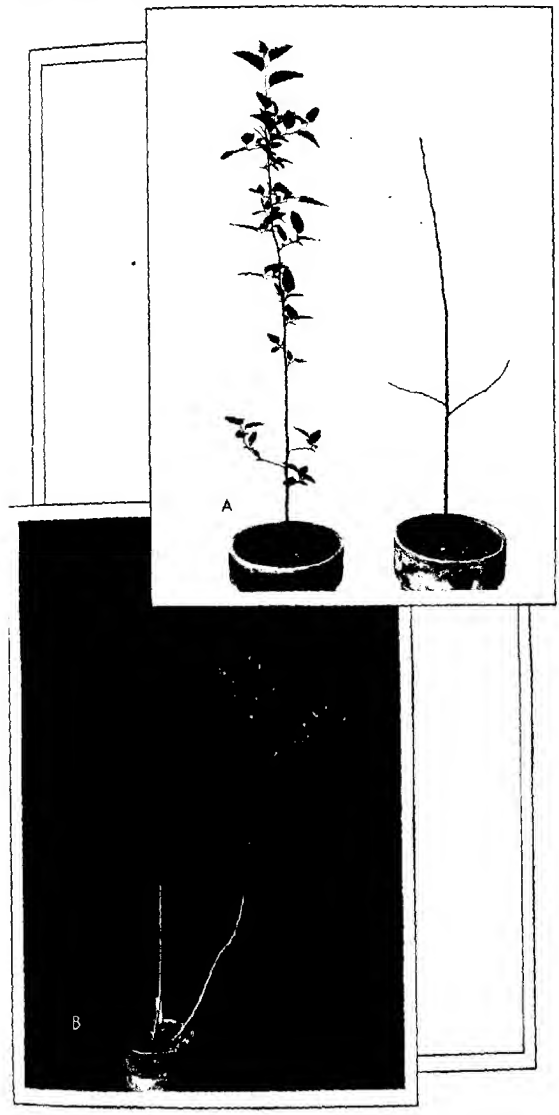
A.—Chilled and unchilled plants of grouseberry, *Viburnum americanum*. The illustration shows two 1-year-old seedlings with the same history, except that the one at the right was kept during the winter in a warm greenhouse at a temperature of 55° to 70° F., while the one at the left was wintered in a cold greenhouse at a temperature of 32° to 40°. When spring temperatures warmed up this coldhouse, the plants in it began to grow, and on April 7, 1914, when the photograph was taken, they had reached the stage shown in the left-hand figure, while the plants in the warmhouse, as illustrated by the right-hand figure, were still completely dormant.

B.—Chilled and unchilled plants of tamarack, *Larix laricina*. These two seedlings, grown from seed procured in Alaska, have had the same history except that the one at the left was wintered in a cold greenhouse at a temperature of 32° to 40° F., the one at the right in a warm greenhouse at a temperature of 55° to 70°. When the photograph was taken, on April 10, 1914, the chilled plant had put out new growth in the warm spring weather, while the unchilled plant still showed only its leaves of the year before.

PLATE 22

A.—Chilled and unchilled plants of wild crab, *Malus coronaria*. The plant at the left had been outdoors during the fall and winter, leafless and dormant, exposed to the frost and cold. The plant at the right had been in the warm greenhouse during the fall and winter at a temperature of 55° to 70° F. When the outdoor, chilled plant was brought into the greenhouse in early spring, it promptly began to put out new leaves and twigs, but the indoor, unchilled plant continued its dormancy. The photograph was taken April 24, 1917.

B.—Blueberry plant with one branch stimulated to growth by cold. The right-hand branch has been stimulated to growth by chilling; the left-hand branch has been kept dormant by heat. For a detailed description of this experiment see p. 152–153.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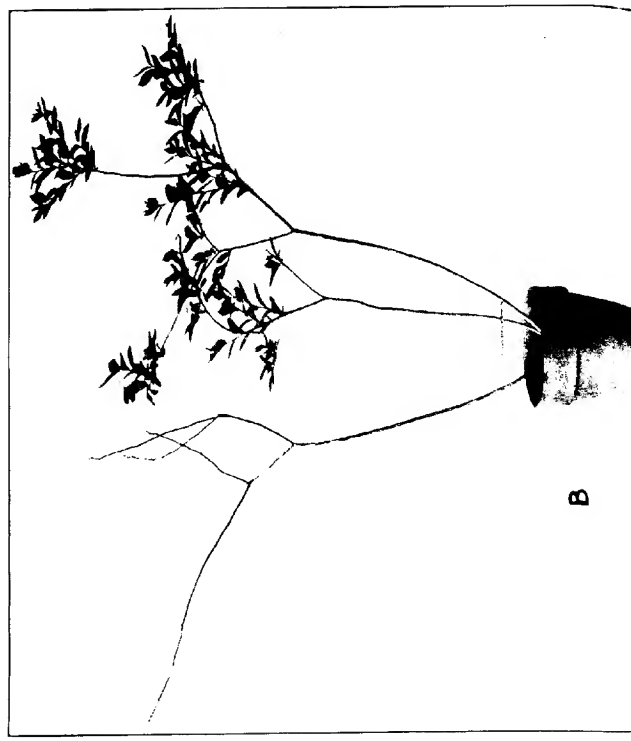
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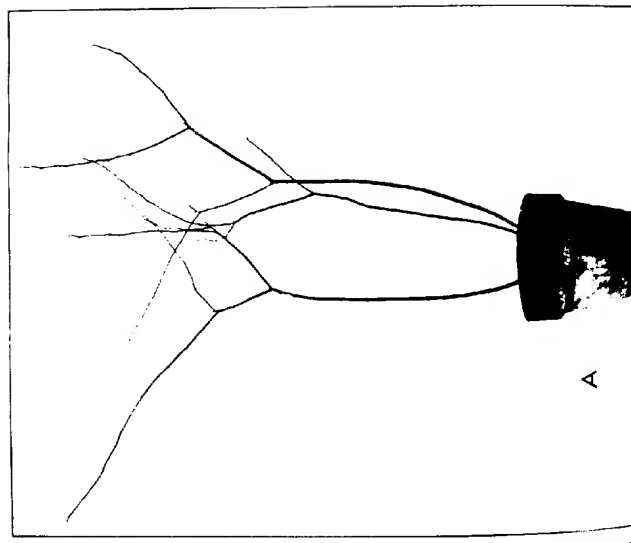
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PLATE 23

Blueberry plant with one branch kept dormant by heat.

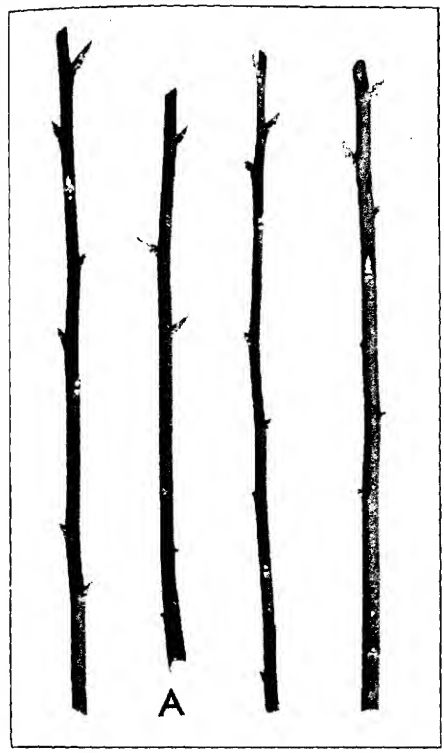
A.--Dormant indoor blueberry plant as it appeared on February 15, 1912. On that date the pot containing the plant was placed on a shelf outside a greenhouse, and a single branch was passed through the glass wall into the warm interior.

B.—Same plant photographed May 21. When spring came, all the outside branches, which had been chilled, burst into normal leaf, while the branch inside the greenhouse, which had been kept warm, still remained dormant.

PLATE 24

A.—Blueberry cuttings starting to grow at 36° F. These cuttings were placed in cold storage while still completely dormant. Although the temperature did not go above 36° F., buds on each of the cuttings finally began to grow. It is to be noted that although growth took place in the buds the other kind of growth which results in the formation of a callus, or healing-over tissue, at the severed base of the cutting is wholly lacking. Callusing can not take place at so low a temperature.

B.—Blueberry plant growing in the dark at 36° F. This plant was in cold storage in the dark in a commercial refrigerating establishment from March 30 to December 4, 1915. The temperature ranged from 33° to 36° F. Some of the plants in this experiment made new growth to the length of 32 mm.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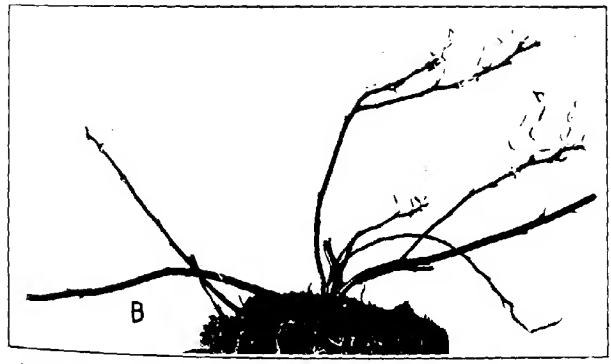
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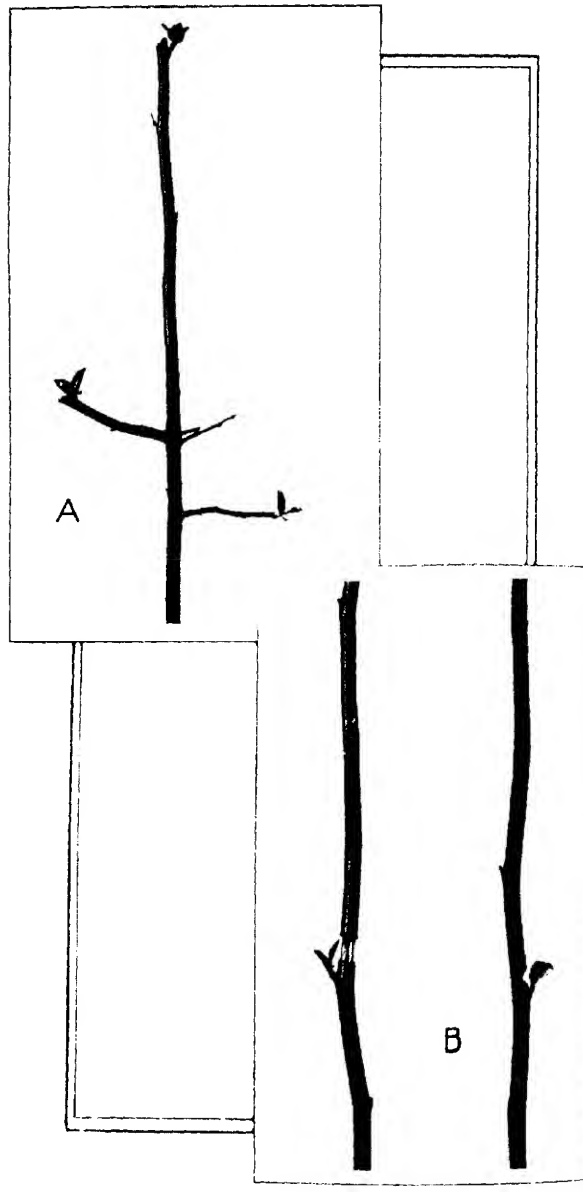
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PLATE 25

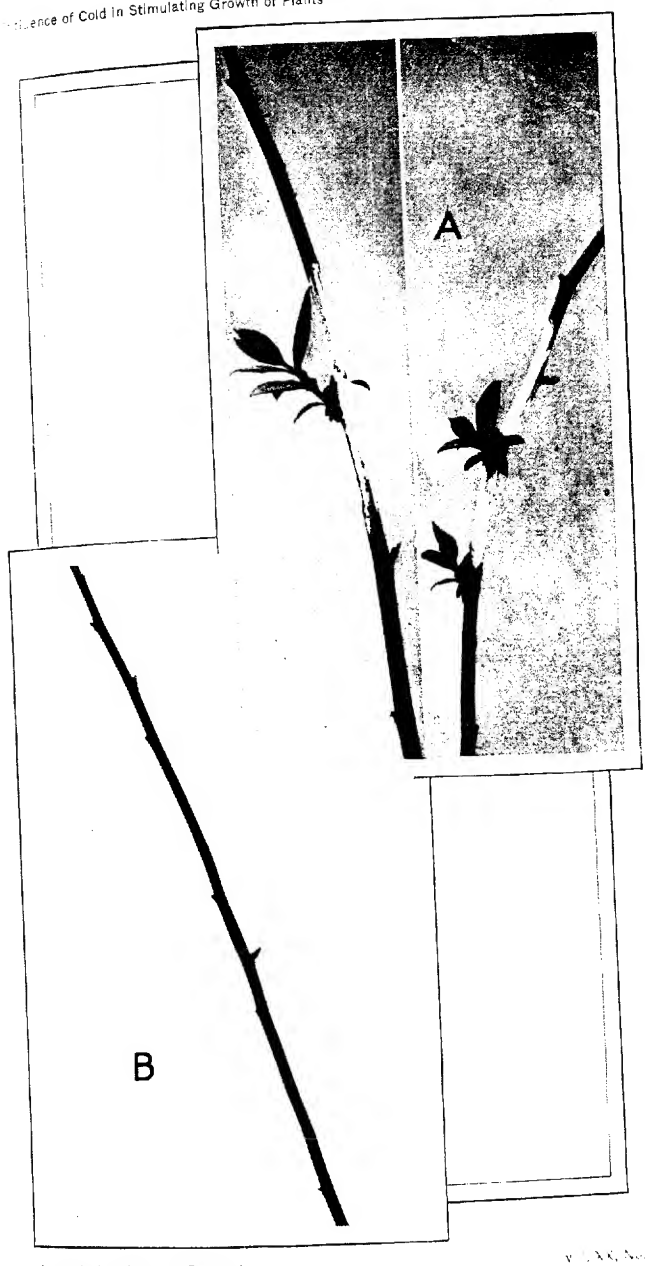
A.—Dormant wild crab stimulated to growth by pruning. This plant had remained dormant in the warm greenhouse during the fall and winter at a temperature of 55° to 70° F. On April 5 three branches were pruned, and on April 24, when the photograph was taken, the uppermost bud on each of the pruned branches had begun to grow. On other, unpruned plants no bud growth had taken place.

B.—Dormant wild crabs stimulated to growth by girdling and by notching the stem. These plants had had the same preliminary treatment as the one illustrated in A—that is, they had been kept in the warm greenhouse all winter, without chilling. On April 4 a ring of bark was removed from the plant shown in the left-hand figure, and the soft cambium was carefully scraped away, down to the hard wood. On April 24, when the photograph was made, the bud next below the girdle had begun to push. The stem of the right-hand plant was notched in early April. The bud next below the notch soon began to grow. The photograph was taken on May 2.

PLATE 26

A.—Dormant blueberry buds stimulated to growth by chalking the stem. This plant was brought into the greenhouse February 4, 1913, to be used in breeding experiments. It flowered, but since it had been insufficiently chilled only a few of the uppermost leaf buds on each stem grew. In order to keep small ants from crawling up the stems and interfering with the pollination experiments the stems were chalked near the middle. The dormant buds in and just below the chalked areas started growing. The photograph was taken April 5, the stems being rechalked over the same areas that were originally chalked. After numerous repetitions of the experiment it was found that if the chalking was done lightly the buds would not grow, but if the stems were rubbed hard in the process of chalking, as commonly happened in the case of very smooth stems, the buds grew. It was the hard rubbing, not the chalk, that stimulated the growth.

B.—Dormant blueberry bud stimulated to growth by rubbing the stem. The photograph, which was taken June 14, 1913, shows a single bud starting into growth on a dormant blueberry plant. The dark area just above the bud is a brown band on an otherwise green stem. It shows the position of a rubbing that was given the stem with a smooth knife handle a few weeks earlier. This bud afterwards grew into a long, vigorous branch, while all the other buds remained dormant.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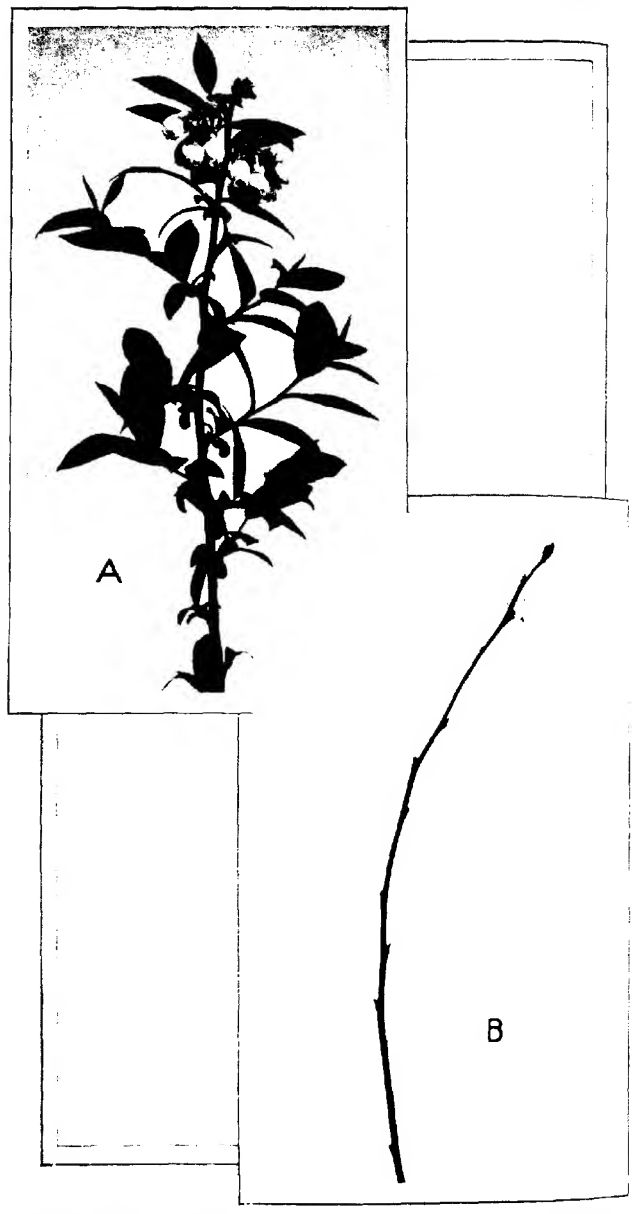
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PLATE 27

A.—Normal spring growth on a blueberry stem. This illustration is from a photograph taken April 24, 1909. In the preceding season the plant had sent up an unbranched shoot. After an outdoor chilling through the winter and early spring it put out flowers and new twigs as shown in the illustration. The fact to be especially noted is that the new growth on this stem took place from numerous buds.

B.—Abnormal spring growth on a blueberry stem, due to lack of chilling. This photograph was taken on May 19, 1913. Growth is taking place from only one bud, the third from the tip. The uppermost bud is a flowering bud, the second a leaf bud. Both are dead or dying. This plant had stood in the warm greenhouse all winter and spring. If it had had the usual two to three months' chilling its starch would have been transformed into sugar and the stem would have flowered and put out new twig growth from numerous buds in the same manner as the stem shown in A.

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PLATE 28

Blueberry leaf exuding sugar from glands interpreted as osmotic-pressure safety valves.

This is a leaf of the highbush blueberry, *Vaccinium corymbosum*. The photograph was taken May 19, 1916. The sugar-secreting glands, sometimes called extra-floral nectaries, are situated in this plant on the back of the midrib and along the margins of the leaf, toward its base. The drops of sugar solution have been wiped away from the glands on the left-hand margin and from two glands on the midrib at the base of the second and fourth lateral veins above the sugar drop shown near the middle of the picture. × 4.

PLATE 29

A plant of bunchberry, *Cornus canadensis*, the seeds of which do not germinate without chilling.

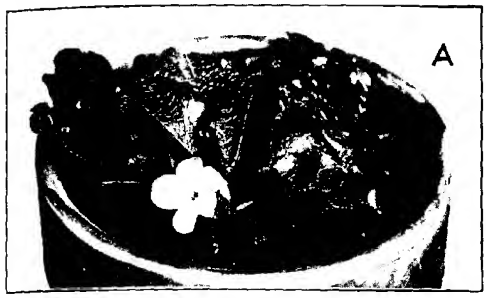
Bunchberry seeds sown October 9, 1912, and chilled during the winter germinated promptly the following spring. Another lot of the same seeds sown on the same date but kept in a greenhouse at a temperature of not less than 55° F. showed no germination in 12 months. These seeds were then chilled for 2 months at a temperature of 35° to 40° F., and when brought back into the greenhouse they germinated within a month. The very healthy plant shown in the illustration grew from one of these long-dormant seeds. The exposure of seeds to winter weather is sometimes practiced by gardeners, but they usually attribute its beneficial effect to freezing, which in all the cases tried in these experiments is unnecessary.

PLATE 30

A.—Trailing arbutus, *Epigaea repens*, flowering sparingly from lack of chilling. This plant of trailing arbutus was grown from seed. In the autumn, when about a year old, it laid down clusters of flowering buds. It was kept in a warm greenhouse all winter, but when flowering time came most of its flower buds were dead and brown. Only a single flower opened.

B.—Trailing arbutus plant flowering normally after chilling. This plant had the same history as the plant described under A, except that it was kept outdoors during the winter and brought back into the greenhouse in the spring. At the age of 14 months, when the photograph was taken, March 27, 1911, the plant was in full flower, healthy and normal.

C.—Blueberry plant forced into flower in September by artificial chilling. This plant was brought indoors in late winter. It made new growth, and during the cool weather of May it laid down flowering buds for the next year, as a blueberry plant ordinarily does in autumn. During the summer, however, the plant was given an artificial winter by chilling it for three months in an artificially refrigerated glass covered frame exposed to daylight. When brought out of the frame, in September, the plant promptly flowered, as shown in the illustration.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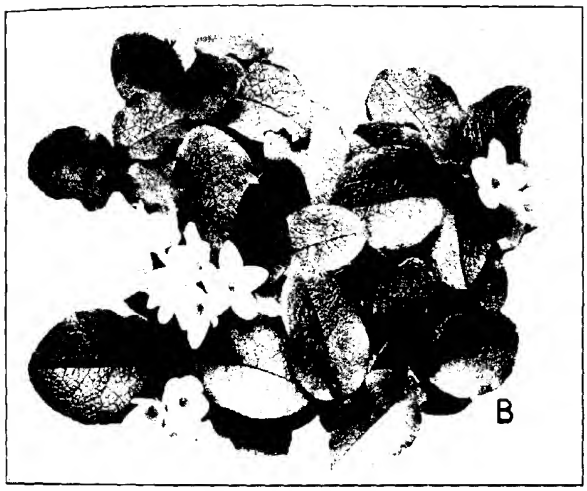
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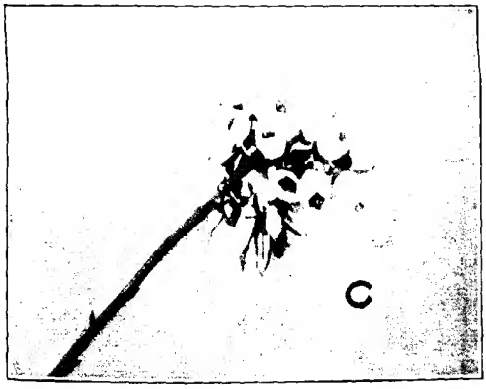
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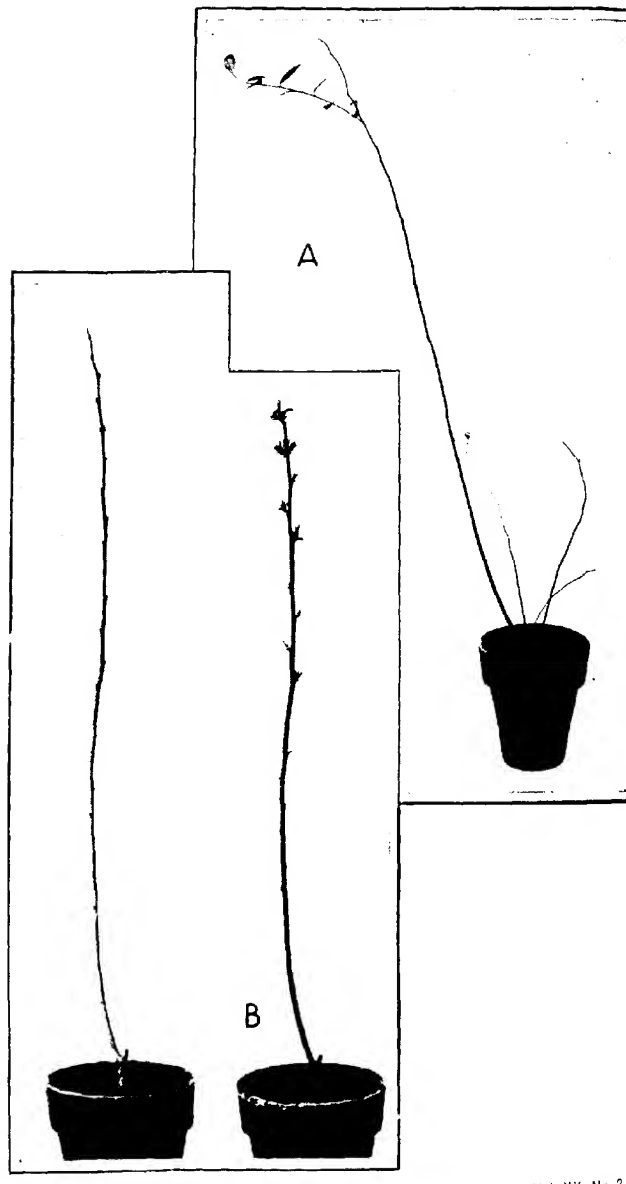
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PLATE 31

A.—Abnormal growth of an unchilled blueberry plant. This plant became dormant in the autumn in a warm greenhouse, and since it was not chilled it continued its dormancy through spring and summer for a period of nine months. Then three of its stems began to die at the tips and, following this, growth began to take place from a single bud next below the dying tip on each stem. For the explanation of this abnormal activity see p. 156. The photograph was taken October 12, 1916.

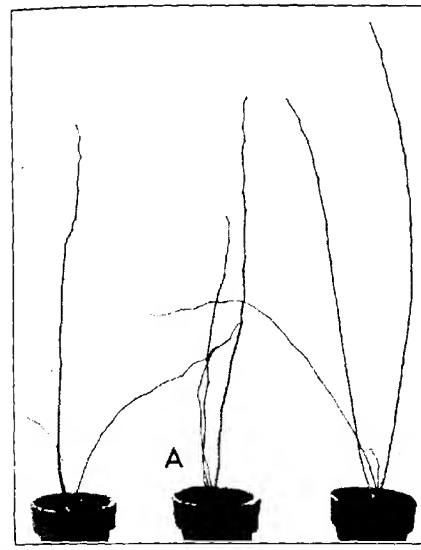
B.—Awakening of long dormant plants by artificial chilling. The illustration consists of two photographs of the same plant. At the left is shown the condition of the plant on December 26, 1916, after more than a year of warmth and dormancy. The figure at the right, from a photograph taken April 27, 1917, shows the appearance of the plant after it had been subjected to artificial chilling for a period of three months and then had been returned to the warm greenhouse. It began to put out new growth from 10 or more of its leaf buds. Even after its extraordinarily long period of dormancy the plant had been brought back to normal activity by a suitable period of chilling.

PLATE 32

Plants brought out of dormancy at a specified time.

A.—Blueberry plants from a lot that had been kept in a dormant condition by warmth for nearly a year. On October 30, 1917, plants from this lot were placed under chilling conditions at a temperature of about 35° F. At the end of a month's chilling eight plants were taken out, repotted, and brought into a greenhouse maintained at a temperature of 50° to 70° F., and after two months' chilling eight other plants were brought out.

B.—Representative plants from each of the two chilled lots described under A, from photograph made January 18, 1918. The plant at the left, which was kept under refrigeration for a month, was only imperfectly chilled, and although it started growing the growth was from abnormally few buds. But the plant at the right, under refrigeration for two months, was adequately chilled and started into growth from many buds in a normal manner. It is evident that by the proper application of this procedure a plant of this nature can be brought into proper condition for shipment and planting on any date desired.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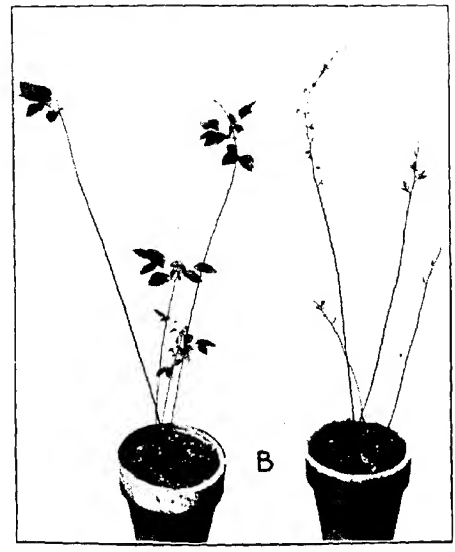
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B

PLATE 33

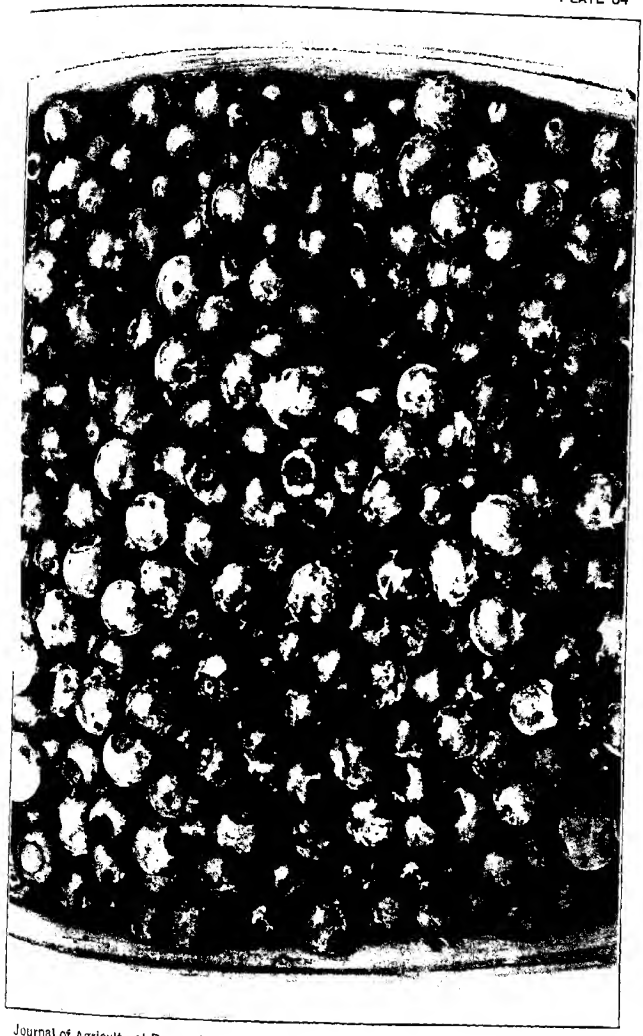
A.—Plantation at Whitesbog, N. J., for the testing of blueberry hybrids. From very carefully selected wild blueberry plants hybrid seedlings are raised in the greenhouses of the Department of Agriculture at Washington. In order to bring them into fruit under favorable outdoor conditions so that selections of the best hybrids can be made for further propagation, the young seedlings are sent to a plantation at Whitesbog, 4 miles east of Browns Mills, in the pine barrens of New Jersey. In the photograph 2-year-old hybrids are shown at the right and 3-year-olds in the row at the left.

B.—Four-year-old blueberry hybrid in full fruit. This illustration shows the vigor, beauty, and productiveness of a hybrid blueberry bush when it is given the proper and peculiar conditions which by its nature it requires for successful growth. From a $\frac{1}{8}$-acre patch of hybrid bushes a yield of berries was secured in 1919 at the rate of 96 bushels per acre. They sold at a little over $10 a bushel, bringing gross receipts at the rate of $966 per acre. In 1920 this planting yielded at the rate of 117 bushels per acre, which sold at a little less than $11 a bushel, yielding gross receipts at the rate of $1,280 per acre.

PLATE 34

The ordinary wild blueberry of New Jersey.

This is a photograph, natural size, of a quart box of wild New Jersey blueberries, rather better than the average. It was taken for the purpose of comparison with the selected hybrid blueberries shown in Plate 35.



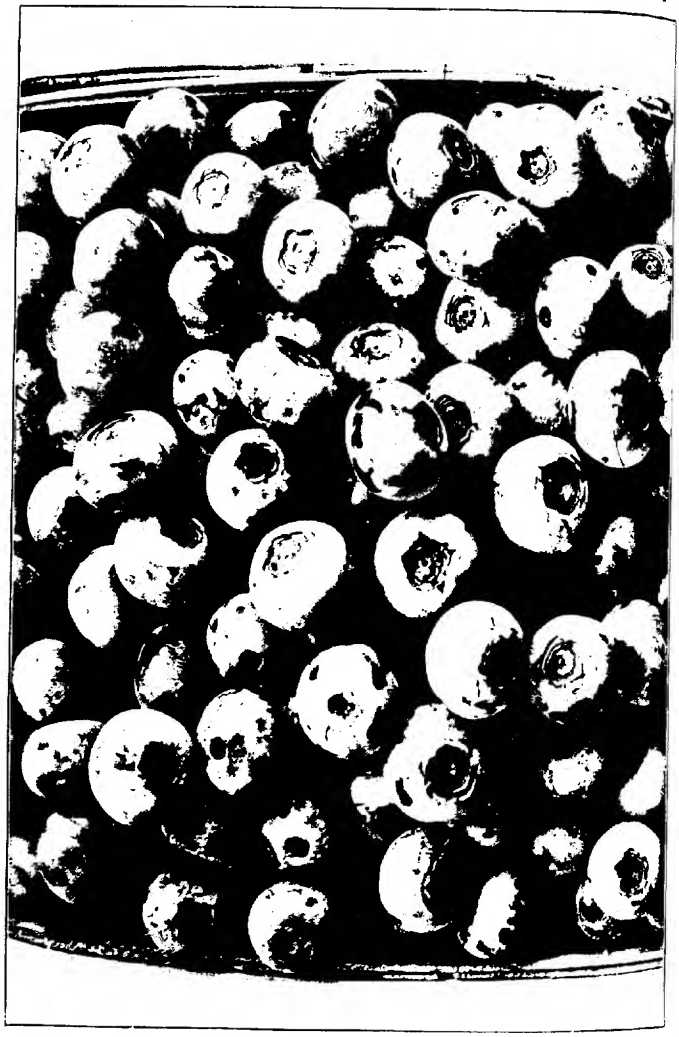


PLATE 35

Fruit of a selected hybrid blueberry.

This illustration shows in natural size a quart box of blueberries from a hybrid produced at Washington and fruited at Whitesbog. The photograph represents the average product of the bush, for it was taken from a clean picking, including the small berries as well as the large ones. Hybrids with berries of still larger size have been fruited at Whitesbog.

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